CELLULAR DEVELOPMENT IN THE LEAF

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INTRODUCTION

The subject of this investigation is the development of cells in relation to the general development of the leaf in wheat. At a certain stage in the development of the leaf cells are being generated from an intercalary meristem; this situation was first accurately described by Lehman (1906). Such a development is essentially different from that of the dicotyledonous leaf in which divisions may be randomly distributed over the leaf or may be restricted to groups of marginal initials. In the Gramineae where the leaf is generally lanceolate or filiform there tends to be a localised meristematic zone at the point of insertion of the leaf on the stem or at the point where the lamina is attached to the leaf sheath. This intercalary meristem produces cells which become incorporated into the body of the leaf and which themselves extend the length and area of the leaf. The time over which the meristem is active is restricted and tends to be confined to the phase of early development. At a certain point, activity in the meristem ceases and thereafter continued growth of the leaf is due to the expansion of the cells which have already been formed. When the meristem is active, cells at different parts of the leaf are at different stages of development. In general, cells are at progressively advanced stages of development as distance from the base of the leaf increases. Clearly this situation resembles, in certain respects, the situation in the apical zones of the root and shoot, and it may be possible to study cellular development in the leaf with the techniques that have already been applied to the root and shoot. On the other hand, since the intercalary meristem is only active over a limited period the study requires an analysis of the growth of the leaf as a whole.

DEVELOPMENT OF THE LEAF

The Primordium

The mode of origin of the leaf primordium in the Gramineae has been the subject of many investigations. Douliot (1891) recognised the primordium as an annular swelling encircling the stem tip, but he considered that this ring gave rise to the leaf sheath and that the lamina grew apically from a terminal cell at the top of the sheath. Bugnon (1924) noted that each new primordium arose on the side of the apex opposite the previous one and that periclinal divisions in the epidermis and the underlying layer spread sideways to give a ring-like protuberance. He considered this ring to give rise to the lamina and said that it possessed a great number of terminal initials along its free edge. Rosler (1928) described the mode of origin of the leaf at the growing point for wheat but thought that the primordium arose solely from the dermatogen. In a similar study on Avena, Kliem (1937) recognised that some of the inner tissue of the leaf base was derived from the 'corpus'. Sharman (1942a) with Zea mays noted that each leaf primordium was initiated by periclinal divisions in the 'dermatogen' accompanied by divisions in the underlying cells. The rapid lateral spread of these divisions causing the characteristic encircling insertion of the primordia. The same author studied many different grasses and cereals (1942b) and described three main types of shoot apex. These were either long, medium or short depending on the numbers of primordia present at any one time. Wheat was classified as having a short apex, there being only one or two primordia at the same time. The problem of which tissue gave rise to the primordia was extensively investigated by Sharman (1945) using many different members of the Gramineae. He found the situation to be similar in all of them and described it for Agropyron repens.

The shoot apex was found to consist of three layers; the dermatogen, the hypodermis and the subhypodermis enclosing a central core. The first two layers each had separate groups of initials while the subhypodermis and the central core shared a third group. The origin of a leaf primordium could first be detected by isolated periclinal divisions on one side of the apex. These divisions may, however, be preceded by divisions in the hypodermis. Further divisions in these two layers produce the crescentic protrusion. The activity of the dermatogen adds to the tissue of the tip and the margins,while the inner tissue is derived both from the hypodermis and the dermatogen but none from the subhypodermis or the central core.

Early Growth of the Leaf

The early growth of the leaf may be considered as apical, and marginal and submarginal initials have been distinguished on several occasions, by Sharman (1942a) Esau (1943) and Mericle (1950) in Zea mays, by Kaufman (1959) in Oryza sativa and by Pray (1957) in Maranta and Philodendron though he failed to find them in Hosta. These marginal initials are in parallel layers and they divide anticlinally during increase in leaf surface. Procambial strands originate in the middle layers and these disturb the original parallel stratification. The lower half of the disk of insertion was considered by Sharman (1942a) to give rise to the internode.

Apical and marginal growth are not distinct processes in the Gramineae as they are in the Dicotyledons and also there are no two stages like the development of the midrib-petiole axis and then the development of the lamina (Esau 1953). Also they are shortlived and intercalary growth from the base of the primordium takes over.

At first there is no distinction between leaf and sheath but when the primordium is still quite small (about 1 cm. in Lolium perenne according to Soper and Mitchell (1956)) a narrow band of small parenchyme cells is differentiated dividing the intercalary meristem in two, the upper half continues to add cells to the lamina while the lower half adds cells in an upward direction to the sheath. Thus the two meristems gradually become further and further apart. The meristem of the sheath is usually active for a longer period than that of the blade and is sometimes capable of a period of dormancy followed by renewed growth; this is not so with the meristem of the lamina (Begg and Wright, 1962). The leaf blade is further demarcated from the sheath by the formation of the ligule, which develops from the adaxial protoderm at the top of the sheath (Kaufman 1959).

Differentiation in the leaf is apparent at a very early age indeed, even before germination as has been shown by Stebbins and Shah (1960) with the stomatal rows of Hordeum vulgare. A similar observation was made by Foard and Haber (1961) who found that when cell division was prevented by irradiating the seeds of wheat, the resultant seedlings had leaf hairs and stomata but that these were abnormal and confined to the apical part of the leaf.

Having the meristem at the base rather than at the apex as in the root, presents special problems in the growth of the leaf. Principle among these is the transport of water and solutes to or from the cells which have been produced above the meristem. This would be impossible if the meristem were completely undifferentiated, however it has been shown by Sharman (1942a) that this is not the case in Zea mays and that the median provascular strand probably has its origin, independent of the rest of the leaf vascular system, at a point in the axis some distance below the insertion of the primordium.

Further, while differentiation in the body of the leaf including the smaller vascular bundles proceeds basipetally from the tip, in the median bundle and the main lateral ones it proceeds basifugally. Esau (1943) noted that since the maturation of the smaller bundles in Zea mays occurred after elongation had ceased, these contained only protophloem and protoxylem.

The grass embryo was described by Barnard (1964) as being highly specialised and containing the primordia of two or more foliage leaves. Also it has a well developed provascular system, the protoxylem elements of which may frequently be lignified. The vascular system was shown to run from the scutellum to the axis and possibly into the first foliage leaf.

The early growth of the leaf may be taken as complete when the intercalary meristem at its base ceases to divide. The cells of the leaf may now be considered to be in an age series with the oldest cells at the apex and the youngest at the base. Further growth of the lamina can only be accomplished by elongation of cells already present. Sharman (1942a) describes the final wave of differentiation as starting at the leaf tip and moving down the lamina, sheath and internode.

The Mature Leaf

The cell types present in the mature leaf may be placed in three main categories; epidermal, vascular and mesophyll. The epidermal cells commonly consist of three main types; long cells, short cells and fibre cells, these being arranged in longitudinal rows their exact formation depending upon the position of the vascular rows. The short cells are often specialised as silica cells or cork cells. The mesophyll cells usually show no differentiation into an upper and a lower palisade and in simple types are compact and quite homogenous.

This is considered by Barnard (1964) to be due to the fact that the leaf usually maintains an erect posture until the differentiation of the mesophyll is complete. A layer of large cells frequently develops in the mesophyll between the vascular bundles; these cells break down as the leaf matures, forming intercellular spaces. This phenomenon has been described by Page (1947). In more complex types, the mesophyll cells tend to be orientated around the vascular bundles. The development of the vascular bundles, with the central and the main lateral bundles differentiating upwards from the axis into the primordium and the smaller ones arising later and differentiating downwards from the tip, has been described above. The anastomosing bundles connecting one vascular strand to another were early described by Strasburger (1891) as consisting of vascular tissue surrounded by a single-layered parenchymatous sheath containing chloroplasts. The main bundles are also contained in one or two sheaths; the inner sheath consisting of small thick-walled cells not unlike endodermal cells and the outer layer made up of large thin-walled cells which are particularly active in starch formation. Barnard (1964) points out that the inner sheath is often difficult to separate from the cells of the bundle proper.

It was recognised by Percival (1921) in wheat that the first leaf in the gramineae may not, in all respects, be the same as subsequent leaves. It is frequently smaller than the others and it is orientated at right angles to the plane of phyllotaxis of succeeding leaves. Also it envelops and protects the axillary bud and may, in shape, somewhat resemble the coleoptile. It is, in wheat, of even width until close to the tip and has a rather blunt apex. This contrasts with the rest of the leaves which are more tapering and are drawn out into long, acuminate points. This last mentioned character of the first leaf gives it an added advantage for the present work.

Since, at a certain stage, the development of the leaf is dominated by the activity of an intercalary meristem, it should be possible to apply the techniques which have already been developed for roots and shoots for the study of cell growth and development. In principle, the technique involves taking successive sections at increasing distances from the active meristem, making observations on individual segments and relating the quantitative data obtained to the numbers of cells in the successive sections. In the root and the shoot, it has been claimed that this technique has limited significance since the variety of the cellular groups is large. It is of some interest that the variety of cellular forms across the gramineous leaf is comparatively limited; the epidermal cells are, of course, distinctive as are the cells of the vascular strands. These, however, are relatively small in number and the dominant component is the mesophyll, whose cells are more or less uniform. The quantitative observations on the successive groups of cells are, therefore, likely to refer primarily to the characteristics of the mesophyll cells.

The investigations that have hitherto been made on cellular development in an intact system have been primarily on the root, and they involve two groups of observations; firstly, observations on the quantitative characteristics of the cell, involving measurements of cell volume, dry weight, fresh weight, protein content, nucleic acid content and the cellular organelle complement of various regions (Brown and Broadbent 1950, Heyes 1960). A second group of observations have been concerned with metabolic changes (Robinson and Brown 1952, 1954; Robinson 1956; Hellebust and Forward 1962). Some of these latter investigations have been directed to elucidating the metabolic mechanisms that are particularly involved in the extension process and these have frequently been concerned with the particular metabolic systems that may be connected with the operation of the phytohormones.

Others have been concerned with the measurements of respiration and of the activities of particular enzymes simply as indices of general metabolic change and particularly with the degree of differentiation of the protein complement of the cell.

Until the work of Preston (1938) on the coleoptile and of Blank and Frey-Wyssling (1944) on the hypanthium of Oenothera, it was considered by many that cell expansion was a process of inflation. However, Preston showed that during the growth of the coleoptile, the dry weight of the wall increased and Frey-Wyssling that in the growth of the hypanthium protein content also increased. These conclusions have been confirmed by more extensive observations on the root and it has been shown that as the cell expands from the meristem to the mature volume the dry weight of the cell may increase tenfold and the protein content to a similar extent. It has also been shown in the root that dry weight increase is due mainly to polysaccharide components of the wall. The increase in protein, although striking, only contributes a small proportion of the total increase in dry weight. The increase in protein during expansion implies an increase in the mass of cytoplasmic components and this is consistent with changes in the nucleic acids and in various cytoplasmic organelles. It has been shown that the increase in protein is accompanied by increases in RNA and DNA in the root and also by increases in the numbers of mitochondria and ribosomes. Increases in the quantity of DNA per cell have also been recorded and this has been attributed to the incidence of endopolyploidy in various parenchyma cells of the root.

In spite of repeated attempts a decisive identification of one or more metabolic systems that are involved in extension has not been made.

On the other hand, it has been shown that the general metabolic pattern changes as expansion proceeds. This has been demonstrated by determinations of the activities of different enzymes and by appropriate analyses of respiration. The position with regard to respiration was confused until it became possible to relate the respiratory activity of different regions of the root to the number of cells they contained. Several workers who measured respiratory activity at increasing distances from the apex of the root observed that, per section, the highest respiratory rate was given by the extreme apical zones and concluded from this that respiration was, in fact, highest in meristematic cells. When these observations were placed on a unit cell basis, however, it was found that the lowest rate per cell is given in the meristem and that the rate increases as the cell expands. The particular instance of respiration is instructive since it illustrates many of the features that were subsequently established with single enzyme systems. When respiration is expressed per unit protein a further significant feature emerges frequently as the cell expands the rate per unit protein increases. Attempts have been made to relate changes in the rate of respiration during expansion to changes in substrate concentration. The relevant correlation has not been established and it seems more probable that the changes in respiration are an expression of corresponding changes in the catalytic systems involved and this interpretation is supported by other results on changes in the activity of single enzyme systems. In terms of this interpretation, changes in the rate of oxygen uptake per cell may be attributed partly to an increase in enzymes consequent on the accumulation of protein and secondly to an increasing proportion of the appropriate enzymes in the whole protein complex.

It may be supposed that the protein that accumulates during expansion is at least partly catalytically active and that the accumulation of protein would, therefore, necessarily involve an increase in oxidising enzymes along with many others. At the same time, the fact that respiration increases per unit protein implies that the proportion of oxidising enzymes in the total protein complex is increasing; thus, in a sense, the development entailed in expansion is at least partly a consequence of increasing differentiation of the protein complement.

Observations have been made with the serial section technique on the activities of single enzyme systems during the expansion process. It has been shown with an invertase system, a phosphatase system, a dipeptidase system, a proteolytic system and a ribonuclease system that activity in all cases is low in meristematic cells and that activity tends to increase as the cells expand. In certain cases, the increase continues until the maximum volume is reached, in others the increase ceases before this stage and, in some cases, not until after it. With the invertase, dipeptidase, protease and ribonuclease systems there is also an increase when the activities are expressed on a unit protein basis and, again the maximum value may be attained either before, after or at the time that maximum volume is reached.

With the single enzyme systems, the most probable interpretation is the same as that which has been proposed in connection with respiration. As the cells expand, there is an increase in total protein and also, for most enzymes, an increase in specific activity per unit protein. This suggests that the proportion of catalytic protein/"inert protein" is also increasing.

In this context, the particular case of phosphatase is of some significance. With this enzyme system although total activity increases as the cells expand, the change on a unit protein basis, is probably not significant. This instance serves to emphasise the general principle involved in cell differentiation as related to enzyme activity. In the root, it has been established that it is the changing proportions of one relative enzyme activity/to another that is the principal characteristic of differentiation. Thus in the meristematic cells the quantitative relationship between the various enzymes is such that differentiation cannot occur. Those cells in which this pattern is maintained, remain meristematic. Further, changes occur in the enzymic activities of the cells cut off from the meristem, enabling them to expand and differentiate. These changes are not only in the total activity of a particular enzyme system or in its activity per unit protein but also in the ratio of activity of one enzyme system to that of another. These arguments are fully developed by Heyes and Brown (1965) who postulated a succession of protein states with each of which different overall metabolic activities and catalytic functions occur. This process is, of course, continuous from the meristematic to the fully expanded cell.

One of the objects of this investigation is to determine whether the enzymic pattern of cell development in the leaf is similar to that outlined above for the root. It has been shown in other organs that development does not cease when the mature volume has been reached.

James (1953) showed that while the terminal oxidase in meristematic and growing zones of the root was probably ascorbic oxidase, in mature regions it is cytochrome oxidase. Brown and Possingham (1957) later made a similar observation and showed that the cyanide resistant system is relatively more abundant in the mature regions than it is in the growing ones. They also showed that specific oxidative capacity changed as the length of the cultured root was traversed. Further, it has been shown that when following expansion the peak protein content has been reached the protein content immediately declines. It has also been shown in the root that various catalytic systems decrease as soon as the protein content decreases. These changes in absolute activity are accompanied by changes in relative activity. During the phase immediately after the mature volume has been reached respiration, invertase, dipeptidase, protease and ribonuclease decrease in activity per unit protein. Thus, while the total protein is decreasing in the early stages of maturity, the proportion of these enzymes in the total protein complement is decreasing. Observations on the phase of maturity are not as extensive as those which have been made on the phase of expansion. Nevertheless, these sporadic observations indicate that progressive changes in quantitative characteristics occur during the phase of maturity and also in the levels of different catalytic systems and that, therefore, the whole metabolic pattern is changing as much during the phase of maturity as it is during expansion.

While this investigation is directed primarily to the development of cells in the leaf, since it involves observations over a protracted period of time, it may also be considered as a contribution to the general study of the gramineous seedling as a whole. In particular, as the phase of development represented by the growth of the first leaf has not received extensive attention. The anatomy and morphology of the grass embryo has been elaborately investigated and several accounts are available which summarise this work. The germination phase has also been considered in detail particularly since the pioneer work of Brown and Morris (1890). These workers made the significant observation that the embryo could be detached from the endosperm and could be induced to grow on synthetic media. They further noted that the normal growth of the embryo was dependent on the release of enzymes from the scutellum and the aleurone layer which converted the insoluble reserves into soluble products, which could be adsorbed by the embryo. Following on this work, the results of a large number of investigations were published on the conditions promoting endosperm mobilisation and the enzymes involved. The actual growth and development of the seedling was not examined in any detail until about thirty years ago. James and James (1940) measured the respiratory activity of the barley embryo during development and showed that this traversed a series of distinctive phases. This was followed by the work of Brown, R (1943a, 1943b and 1946) who demonstrated a distinctive lag phase in the early development of the seedling of barley; this was followed by a period of exponential growth and a similar exponential increase in protein content and dry weight.

Ingle, Beevers and Hageman (1964) investigated the metabolic changes associated with the germination of corn and followed changes in fresh weight, dry weight, nitrogen fractions and nucleic acids. They found that a loss in dry weight occurred in the embryo axis over the first 24 hours indicating that the use of endogenous substrate occurred before the transport of reserves from the endosperm. They also found that slight early growth occurred without the synthesis of DNA or cell division. Ballard (1964) also noted the occurrence of extension growth before cell division in the embryos of various cereals.

After the early work of Went (1930) on the phototropic responses of the coleoptile, which led ultimately to the isolation of auxin, a large number of investigations were undertaken on the development of the coleoptile. This phase of seedling growth cannot be reviewed here but some important aspects may be mentioned that are relevant to the present work. Firstly that during the early growth of the coleoptile, both cell division and cell extension are involved. This phase lasts until the coleoptile is about 1 cm. in length and all further growth is through cell extension only. Further, that as soon as the coleoptile begins to extend, the first leaf, which starts as a primordium in the embryo, begins to grow and eventually breaks through the apex of the coleoptile when the latter has reached its maximum length. Subsequently, various quantitative aspects of the coleoptile have been studied and it has been shown by a number of workers that during the expansion phase there are increases in wall constituents, proteins, etc. Wright (1961) found that the net increase in protein content was greatest when cell division had ceased. RNA content decreased when the cells had reached their final volume but DNA content continued to increase.

It was suggested that this was due to endopolyploidy. This phenomenon has also been identified in roots, Heyes (1960) found that cell expansion in the pea root was accompanied by a fourfold increase in RNA and a twofold increase in DNA per cell. This work, however, was not continued into the mature region of the root. McLeish and Sunderland (1961) and Sunderland and McLeish (1961) using the same serial segment technique as employed by Heyes found, both by chemical and photometric methods that DNA content continued to increase after cell expansion was complete and they concluded that this was due to an increasing proportion of 4C nuclei and a corresponding decrease of 2C nuclei.

A large amount of work has been performed in recent years on the nucleic acids of gramineous seedlings. The principal focus of this work, however, has been on methods of extraction and estimation rather than on the significance of the nucleic acids with regard to development and differentiation. A comprehensive survey of this literature is, therefore, beyond the scope of this introduction (some of the prominent workers in this field may, however, be mentioned, e.g. Bergvist (1956 and 1957) on the acid soluble nucleotides of wheat, barley and oats; Cherry and Hageman (1960 and 1961) on the nucleotides of corn seedlings, Ingle (1963) on barley nucleotides; Keys (1963) on wheat nucleotides and Ingle and Hageman (1965) on nucleic acid metabolism in corn).

The development of the first leaf of wheat has not been studied in any detail but de Ropp (1945 and 1946) studied the development of the first leaf of rye both with embryos cultured on agar and with intact seedlings. This worker found that the growth of the first leaf on agar was limited and further that no cell divisions occurred under these conditions.

With leaves from intact seedlings de Ropp found that most of the growth of the leaf occurred from the base, from the basal 0.3 mm. of a 1.2 mm. primordium. During the course of these experiments he had occasion to remove the coleoptile in order to place marks directly on the first leaf and he recorded that this treatment had no effect on the growth of the leaf.

Rhodes and Yemm (1963) investigated the synthesis of proteins and the development of chloroplasts in the first leaf of barley. They noted that 70 to 80% of the leaf protein and 20 to 30% of the RNA could be found in the chloroplasts. With illuminated leaves, the RNA peak per leaf was found at five days and the protein peak at seven days. RNA declined fairly rapidly after the peak value but protein did not. DNA content also reached a maximum at seven days and then remained constant. When the seedlings were kept in the dark, it was found that the nucleic acids gave similar values to those found in the light but the protein content was considerably lower and declined after reaching the maximum value. It would appear that the maximum values for nucleic acid and protein content were recorded at approximately the time of cessation of cell extension. Rhodes and Yemm (1966) studied the development of chloroplasts and photosynthetic activity in young first leaves of barley. The seedlings were germinated and allowed to grow to a length of 3 to 4 cms. in the dark and were then transferred to the light. It was found that there was an appreciable effect of light on the carbon dioxide exchanges after three to four hours. Also, a marked increase in the efficiency of the pigment system was found between the value for photosynthesis per unit chlorophyll at 12 hrs. and at 84 hrs. after the start of illumination.

The authors concluded that their results were consistent with the theory that the protochlorophyllide formed in the dark was photoreduced to chlorophyllide in the light and that this was then more slowly converted to chlorophyll by enzymic phytylation.

A comprehensive study of the growth of the wheat plant was made by Williams (1960) and Williams and Rijven (1965). The first paper deals primarily with the morphological development of the leaves and their relative growth rates, while the second gives a detailed account of the changes in various components, e.g. dry weight , protein content, and nucleic acid content, with the age of the leaf. Figures are given for several leaves but the study was concentrated on the fourth leaf. With this leaf, it was found that RNA content rose to a peak slightly before the leaf reached its maximum size and then declined sharply. Protein content rose to a peak slightly later and did not decline. Although the leaves were occasionally cut into halves or quarters for the estimation of some constituents most of the work was performed on whole leaves and this, of course, only provides evidence on the average pattern of growth throughout the leaf. The material used by Williams was all preserved in alcohol and no attempt was made to investigate the changing metabolic characteristics with age.

From the literature available on the gramineous leaf, it appeared that there was a lack of information concerning the development of the cells of the leaf with age and the metabolic changes characterising this. Until fully mature, the leaf can be divided into arbitrary zones of meristem, expansion and maturation.

Work on the whole leaf obscured the frequently great differences between one end of the leaf and the other and is thus of limited value in following the developmental changes of the constituent cells. Further, Richards (1934) points out that work using successive leaves for the study of physiological change in relation to leaf age is difficult to interpret and that the results should be treated with caution.

As indicated earlier, the main subject of this investigation has been the average development of cells in the leaf from the meristem, through the fully expanded state and including some of the mature phase. For reasons that have been given below the observations have been made on the first leaf of wheat and the general purpose of this investigation has been to follow development from the intercalary meristem. This meristem, as shown earlier, is only transitory and the investigation, therefore, necessarily involves an examination of the development of the leaf as a whole. The purpose of this being to discover the period of time for which the meristem is active under the experimental conditions, and also to delimit the time during which expansion continues after the meristem has ceased to divide. The first part of this thesis is, therefore, devoted to observations on the development and pattern of growth of the leaf as a whole. The investigation of the development of the cells is based on a technique analagous to that used with roots. While the meristem is still active the leaf is dissected along its length into serial 5 mm. segments. It was found that it was impracticable to continue this pattern of observations after meristematic activity had ceased, since the length of the leaf becomes such that a complete dissection would have involved an unmanageably large number of segments.

Nevertheless, the same general principle was adhered to although the segments taken were restricted to specific areas of the leaf. Thus, the observations on cellular development fall into two groups: the first being taken while the meristem was active and which involves successive segments taken along the whole length of the leaf; the second group are prolonged over a much longer time period starting while the meristem is active and continuing through the period of cessation of division and extension. Only four 5 mm. segments were taken in this second group, three were successive segments taken from the base and one being subapical. The three basal segments were chosen to show the progressive changes in the meristem from the actively dividing state to the stage where all the cells are fully expanded. The subapical segment was chosen to show the progress of development of cells which had been formed early in the life of the leaf.

The observations that were made on the segments in the two groups of experiments were based on the type that had already been made on the root. The observations themselves may be arbitrarily divided into two sections: the first designed to show changes in the quantitative character of the cells and the second to show changes in the metabolic characteristics. The first section involved measurements of fresh weight, dry weight, total nitrogen, protein content, chlorophyll content and in some cases RNA and DNA contents. Determinations of the cell numbers of the segments were also made in order to relate this data to cellular development. The study of metabolic characteristics involved measurements of respiration rate, the activities of an invertase, a phosphatase, and a protease system and, in certain connections, measurements were made of photosynthetic activity.

The general experimental design involved establishing a population of seedlings with a standard mean length of first leaf, taking samples from these at intervals, removing the first leaf and dissecting it as the experimental situation required, and finally making the appropriate determinations on these segments. At the same time, a parallel group of segments was assembled for determinations of cell number and the two groups of data from corresponding segments were used for the estimation of a series of values per unit cell.

MATERIAL AND METHODS

The choice of experimental material was governed by a number of considerations. Leaves were required throughout the year and it was, therefore, necessary to use material which could be grown easily under artificial conditions. Secondly, it was necessary to have a continuous supply of standardised material and, thirdly, the material had to be such that it could be obtained in quantity. The possibility of using large leaves propagated from bulbs, corms or rhizomes was considered but it appeared to be exceptionally difficult to obtain reproducible material in quantity from these. The most attractive sources were plants from various species of the Gramineae; of these, the cereals were considered to be preferable since they generally had the larger and more easily manipulated leaves. In a preliminary series of observations, the relative merits of barley, oats, wheat and maize were examined. Despite having less uniform germination than barley and oats, wheat was ultimately chosen as the experimental material; the principal reason being that when the seeds of wheat are germinated the shoot may grow vertically upwards without distortion. With oats and barley the glumes, lemma and palea are persistent and the shoot only emerges after growth between these and the caryopsis. As a result, with these two species the shoot is usually curved when it emerges. Maize was considered unsatisfactory since, with the samples available, germination and seedling growth were very uneven.

All the observations are based on the first leaf that emerges through the coleoptile, the second and subsequent leaves were found to be less satisfactory, since variability with them is greater than with the first leaf and their use would have required a considerably longer growing period.

Also, during early growth, the second and subsequent leaves may be distorted through being enclosed within the first leaf. For the same reason, it was impossible to measure the growth of these leaves. The ability to measure the length of the leaf and the absence of mechanical distortion is important since the experimental design required the dissection of leaves of known age and length into successive 5 mm. segments.

General Experimental Design

As indicated in the introduction, the experimental design involved two experimental series, which in the rest of this thesis are distinguished as Series 1 and Series 2. In the first series, after the establishment of the intercalary meristem, serial segments are taken from the base of the leaf to the apex. Observations on the quantitative and metabolic characteristics of these segments were made and the results related to the number of cells each segment contains. In this series, for reasons given in a later section, two sets of leaves at 72 and 96 hrs. after germination were used. In the second series, the development of cells in different regions of the leaf was studied over a prolonged period. In this series, it was impracticable to dissect the entire leaf into serial segments, so four segments were taken from the leaves at 24 hr. intervals from 3 to 10 days after germination. Three of the segments were taken serially from the base of the leaf and one from a subapical position. The selection of the material was based on the results of preliminary experiments on the general characteristics of growth of the leaf.

Subjects of Investigation

The subjects of investigation and the techniques used may be described under the following headings:

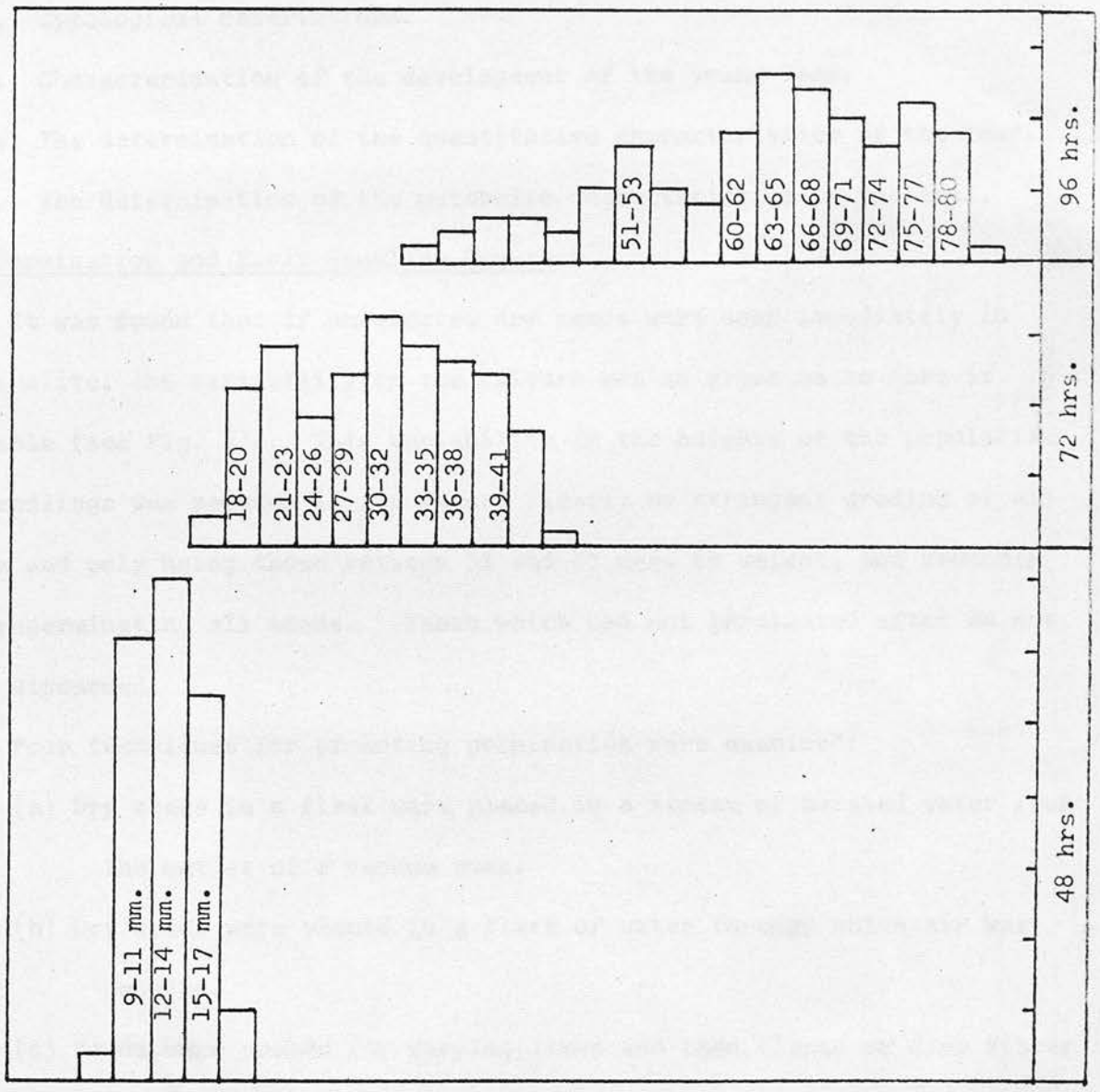


Fig.1 Histogram showing the lengths of first leaves in seedlings from unselected, dry seeds under growth conditions of Series 1. Each division on the right-hand side of the diagram represents 5% of the population.

1. Germination and early seedling growth.
2. Selection of leaves of standard length and the preparation of segments.
3. The anatomy of the leaf.
4. Cytological observations.
5. Characterisation of the development of the young leaf.
6. The determination of the quantitative characteristics of the leaf.
7. The determination of the metabolic characteristics of the leaf.

1. Germination and Early Seedling Growth

It was found that if unselected dry seeds were sown immediately in Vermiculite, the variability in the culture was so great as to make it unusable (see Fig. 1). This variability in the heights of the population of seedlings was reduced in two ways: firstly by stringent grading of all seeds and only using those between 55 and 65 mgs. in weight, and secondly by pregerminating all seeds. Those which had not germinated after 24 hrs. were discarded.

Four techniques for promoting germination were examined:

(a) Dry seeds in a flask were placed in a stream of aerated water from the outlet of a vacuum pump.

(b) Dry seeds were placed in a flask of water through which air was bubbled.

(c) Seeds were soaked for varying times and then placed on damp filter paper.

(d) Dry seeds were placed immediately on damp filter paper.

The fourth procedure gave the most satisfactory results, since it gave more uniform germination and, therefore, yielded the highest percentage of seedlings at the same stage of development.

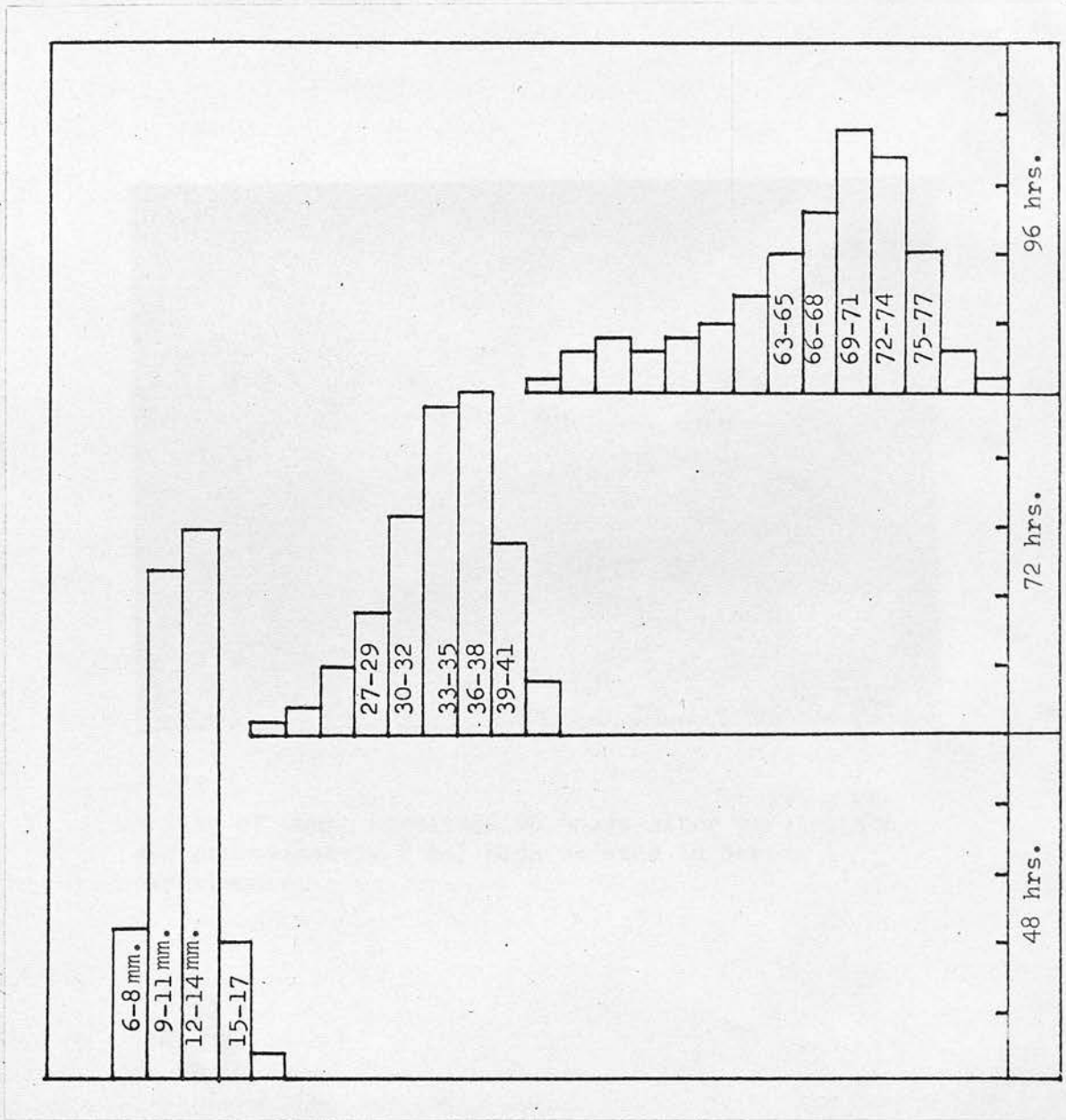


Fig.2 Histogram showing the lengths of first leaves in seedlings from selected, pre-germinated seeds as grown for Series 1. Each division on the right-hand side of the diagram represents 5% of the population.

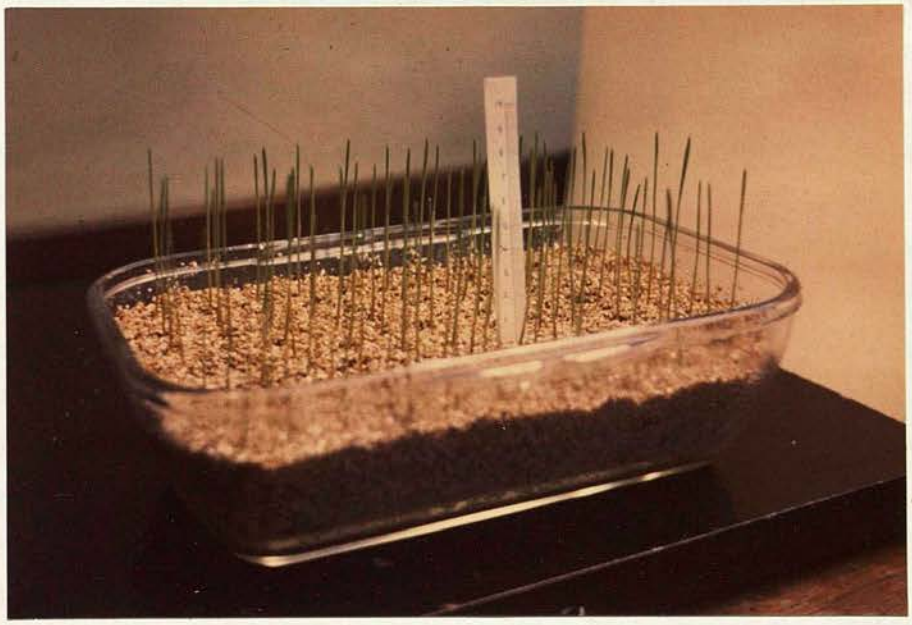


Plate 1

A dish of wheat seedlings 96 hours after germination and approximately 7 cm. high as used in Series 1 experiments.

In the standard procedure all seeds were surface sterilised in methylated spirits for 10 mins. and 10% calcium hypochlorite for 5 mins. The sample was then washed with distilled water and placed in a single layer on filter paper in a Pyrex dish. Each dish carried about 200 seeds which were supplied with 40 mls. distilled water to which had been added 25 ppm. streptomycin sulphate. After 24 hrs. at 22.5°C in the dark the seeds were examined and all those which had just germinated were transferred to damp Vermiculite. The criteria of selection were that the radicle should have emerged and the plumule have just split the testa. The Vermiculite was carried in a Pyrex dish, 2 litres of Vermiculite being mixed with 700 mls. tap water. The dimensions of the dish were 26 x 18 cms. and the depth of the Vermiculite approximately 6 cms.

The germinated seeds were planted individually in rows with the embryo pointing upwards. In this way the shoot grows vertically upwards without distortion and the height of each may be measured on successive days. The dishes were then placed in a constant environment chamber. In Series 1 the conditions of growth were as follows; temperature 22.5°C, daylength 12 hrs. and the light intensity at the dish surface was 540 ft. candles. In Series 2 the temperature was 25°C, the daylength 18 hrs. and the light intensity at the dish surface was 1,600 ft. candles.

In Series 1 measurements of the leaves were made at 48, 72 and 96 hrs. after germination. At these times the length of the stem was found to be negligible, thus the base of the seedling could be taken as the base of the leaf. The population of plants was found to be satisfactorily uniform when the above procedure was applied. The results of a growth experiment of material used in Series 1 is shown by histograms in Fig. 2.Plate 1 shows the appearance of a dish of wheat at 96 hrs.

As the growth period was short in Series 1 it was not found necessary to add nutrient solution to the Vermiculite and the plants were watered only on the third day at the rate of 300 mls. of tap water per dish.

In Series 2 the measurements of first leaf length were taken from 3 to 10 days at daily intervals. Measurements were again taken to the base of the seedling but from 5 days onwards the growth of the stem was appreciable and it was necessary to remove the coleoptiles from a sample of seedlings to find the mean length of the stem on each day. The appropriate correction was then made for the lengths of the first leaves of the rest of the population. After about

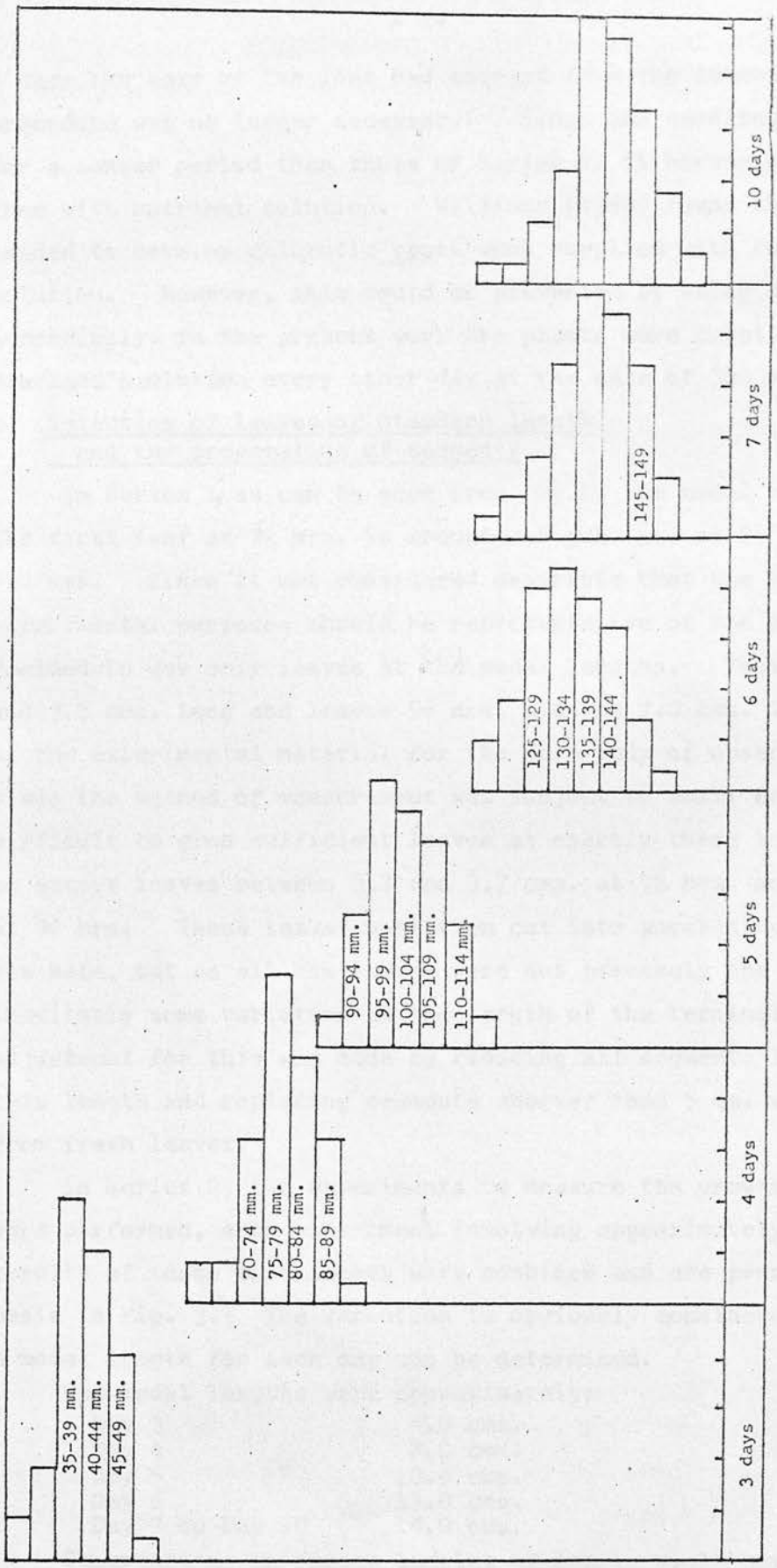


Fig.3 Histogram showing the lengths of the first leaves of the seedlings used in Series 2. Each division on the right-hand side of the diagram represents 5% of the population.

7 days the base of the leaf had emerged from the coleoptile and this procedure was no longer necessary. Since the seedlings were being grown for a longer period than those of Series 1, it became necessary to provide them with nutrient solution. Williams (1960) found that wheat seedlings tended to develop chlorotic spots when supplied with full-strength Hoagland's solution. However, this could be prevented by using half-strength solution. Accordingly, in the present work the plants were supplied with half-strength Hoagland's solution every other day at the rate of 300 mls. per dish.

2. Selection of leaves of standard length and the preparation of segments

In Series 1, as can be seen from Fig.2, the modal value for the length of the first leaf at 72 hrs. is around 3.5 cms. and at 96 hrs. it is around 7.0 cms. Since it was considered desirable that the leaves used for experimental purposes should be representative of the population, it was decided to use only leaves at the modal lengths. Thus leaves 72 hrs. old and 3.5 cms. long and leaves 96 hrs. old and 7.0 cms. long were selected as the experimental material for the main body of observations in Series 1. Since the method of measurement was subject to small errors and it was also difficult to grow sufficient leaves at exactly these lengths, it was decided to accept leaves between 3.3 and 3.7 cms. at 72 hrs. and between 6.8 and 7.2 cms. at 96 hrs. These leaves were then cut into successive 5 mm. segments from the base, but as all the leaves were not precisely the same length, there was inevitably some variation in the length of the terminal segment. An adjustment for this was made by reducing all segments longer than 5 mm. to this length and replacing segments shorter than 5 mm. with terminal segments from fresh leaves.

In Series 2, two experiments to measure the growth of the leaf with time were performed, each experiment involving approximately 100 seedlings. The results of these experiments were combined and are presented on a percentage basis in Fig. 3. The variation is obviously considerable but, nevertheless, a modal length for each day can be determined.

The modal lengths were approximately:

Day 3	4.0 cms.
Day 4	8.0 cms.
Day 5	10.0 cms.
Day 6	13.0 cms.
Day 7 to Day 10	14.0 cms.

Observations on random samples of leaves would have been undesirable but it was considered that in Series 2 a greater variation in length could be accepted than in Series 1, since the emphasis here was on change with age.

Therefore leaves within 4 mms. of the approximate modal length above for the particular day were accepted for experimentation.

Preparation of segments:

Seedlings of the appropriate sizes and ages were removed from the Vermiculite and their shoots excised as close to the seed as possible. Further manipulations of the shoot were carried out on the stage of a Zeiss dissecting microscope. The coleoptile was removed using a surgical tenoteny knife and a mounted needle with a diamond-shaped head. The first leaf was then detached at the position of the ligule which could be identified at an early age by a faint ring marking the position at which hairs are growing on the inside of the curled leaf. At 72 and 96 hrs. the position of the ligule was within 1 or 2 mms. from the base of the shoot. Finally the second leaf was removed from within the base of the first leaf. It was found that these operations had to be performed with great care to avoid damage to the first leaf. Each leaf, as it was isolated, was placed on damp filter paper and left there until a sufficient number had been accumulated. The dissection of the leaf into successive segments was performed on a simple cutting block consisting of a copper plate bent sharply at right angles at one end, spacing blocks 5 mms. in length being soldered to the vertical face. The leaves were usually handled in batches of five and the segments were cut by sliding a razor blade down the ends of the spacers. The basal segments were removed first and subsequently each successive segment. All the segments could not be cut simultaneously since some of the leaves tended to curl at the base after excision and the individuals had to be differently aligned in each batch.

Dissecting the leaves was inevitably slow and only small samples could be used for particular determinations. If larger samples had been assembled significant changes might have occurred in some segments during the time of preparation of the sample.

The basal segment is considered as segment 1 and the segments are numbered serially up to the apical one which at 72 hrs. is segment 7 and at 96 hrs. is segment 14. In Series 2 only four segments were taken on each day of the experimental period, three of these were taken serially from the base and one from a subapical position between 1.0 and 1.5 cms. from the apex. The three segments from the base are referred to as segments 1, 2 and 3 and the subapical one as segment 4. In the text the word segment used in this context is abbreviated to the letter S, thus segment 1 is referred to as S1 and so on.

3. The anatomy of the leaf

An anatomical investigation was undertaken on leaves at the modal length 48 and 72 hrs. after germination. This was based on microtome sections of wax-embedded material. The 48 hr. leaves were cut into two segments, the basal one of 5 mm. and the apical one approximately 7 mm., the 72 hr. leaves were cut into successive 5 mm. segments. The tissue was fixed in formalin: acetic:alcohol (1:1:3) and after embedding the sections were cut at 10 μ on a microtome and stained with Delafields Hematoxylin. The embedding and staining schedules followed were those of Jensen (1962). Both transverse sections (T.S.'s) and longitudinal sections (L.S.'s) were cut from each segment. The T.S.'s being cut from the beginning, middle and end of each segment. The coleoptile was found to be useful for the support of the material and was therefore not removed. Due to the curled-up nature of the leaf, the L.S.'s necessarily cut through the leaf several times in each section. A photographic survey of the sections was made using a Zeiss photomicroscope, serial photographs being taken along the length of each successive segment. From these, a composite reconstruction of the entire leaf could be built up.

The techniques described above were also used when sections were taken through the region of the ligule when the longevity of the meristem was being investigated.

4. Cytological observations

The material used consisted of leaves at the modal lengths 24, 48 and 72 hrs. after germination. The observations are all based on the Feulgen technique and are derived from two methods of maceration, which may be called the hard and the soft squash techniques. In all cases, the same initial procedure was followed.

The tissue was fixed in acetic:alcohol (1:3) for 15 mins. and subsequently washed in cold water for 2 mins. and tepid water for 1 min. After washing, the tissue was treated with normal hydrochloric acid (HCl) for 8 mins. at 60^{o}C., washed for 5 mins. each with two changes of distilled water and immediately transferred to leuco-basic fuchsin solution for 45 mins. or until deeply stained. The material was then washed in sulphur dioxide water and finally squashed in 45% acetic acid. The leuco-basic fuchsin and sulphur dioxide water solutions were made up according to the method of Darlington and La Cour (1960).

In the normal Feulgen procedure the material is first macerated by tapping with a brass rod, the cover slip is then applied and the preparation squashed into a single cell layer. This is basically the technique described here as the 'hard squash technique', though in this work the material was first teased apart with mounted needles. From such preparations the distribution of mitoses along the length of the leaf and the relative number of mitoses in different segments could be assessed. In this method, the cells of individual rows were displaced from each other and when it was required to preserve the continuity of the rows a different technique, the 'soft squash technique', was used. Here after the material had been carefully teased apart, the coverslip was applied with only gentle pressure. In such preparations the cells are not compressed into a single layer but the individual rows are preserved and can be observed at different focal depths.

As indicated above, observations were made with 24, 48 and 72 hr. leaves, the 24 hr. leaves were small enough to be treated whole, the 48 hr. leaves were cut into two segments the basal one being 5 mm. long and the apical one approximately 7 mm. long. The 72 hr. leaves were dissected into seven successive 5 mm. segments.

In one instance, with a 24 hr. leaf, the soft squash technique was used after staining with the periodic acid-Schiff reagent of Jensen (1962). This procedure was used to stain the thickened xylem vessels that were observed at the base of the leaf.

5. Characterisation of the development of the young leaf

At an early stage in the investigation it was found necessary to undertake some preliminary observations to determine the distribution of growth in the leaf at different ages. The general principle used was that of placing marks with Indian ink at various positions on the leaf and subsequently measuring the displacement of these marks from each other.

The seedlings used in each experiment were individually and carefully removed from the dish of Vermiculite and placed on the stage of a Zeiss binocular dissecting microscope, In order to protect the roots from dehydration these were wrapped in damp absorbent paper. In early experiments slitting the coleoptile in the positions where marks were required, was tried. This was found to be an improvement on the complete removal of the coleoptile but the most satisfactory/ (Continued overleaf)

method was found to be the cutting of small 'windows' in the coleoptile to expose the leaf for marking. The instruments used in the dissection of the coleoptile were a surgical tenoteny knife and a mounted needle with a diamond-shaped head. The marks were made with a solution of Indian ink that had been concentrated by evaporation and were applied with fine glass capillary tubing. In the basal area of the leaf, the tissues were damp and soft and great care had to be taken to avoid damage. Any damage to the surface of the leaf in this region was found to be greatly enlarged after 24 hrs.' growth. Because of the surface moisture, it was found advisable to blot the leaf with soft absorbent paper before applying the marks, otherwise the ink had a tendency to spread and a very diffuse spot resulted. The marks were placed as accurately as possible under the microscope using a millimetre scale. Some difficulty was experienced in determining the exact position of the base of the leaf particularly with the 24 hr. seedlings. After marking, the seedlings were carefully replanted in fresh Vermiculite and replaced in the constant environment chamber.

6. The determination of the quantitative characteristics of the leaf

a) Cell number determination

The cell number determinations were made using the technique of Brown and Rickless (1949), on each occasion five segments were macerated in 5 mls. of 5% chromic acid. As most of the segments were curled, air was trapped within them and they floated on the surface of the acid. Various methods of infiltrating the segments under vacuum were tried in order to submerge them. However, none were found to be wholly satisfactory and it was found preferable to allow the segments to float on the surface of the macerating fluid for three to four days. At the end of this time the cells were separated from one another by passing the suspension through a hypodermic syringe with a wide bore needle. It was found that some adjustment was necessary in the time of acid treatment according to the maturity of the cells in the segments. The larger, more mature, cells were generally more susceptible to break-up in the maceration treatment than the meristematic cells. The density of the cells in the suspension was measured on a haemocytometer slide having a grid volume of 3.2 µl. The cell numbers were calculated from the means of six grid counts per suspension.

b) Fresh and dry weight determinations

For fresh and dry weight determinations samples of five segments were used. For fresh weights the segments were carefully blotted, placed immediately in a glass-stoppered tube of known weight, and weighed to the nearest 0.1 mg. Subsequently the dry weight was obtained by placing the open bottle in a drying /(Continued overleaf)

oven at 90°C for 48 hrs. The material was then reweighed, again to the nearest 0.1 mg. In Series 2 a balance weighing to the nearest 0.01 mg. was used.

c) Nitrogen determinations.

(i) Total nitrogen.

Total nitrogen was estimated by the Nessler technique as modified by Vanselow (1940). A sample of five segments was placed in a micro-Kjeldahl tube which was in the form of a test-tube with a constriction near the base. The constriction prevented loss due to 'bumping'. 0.3 mls. of digest acid were added to each tube together with a few Carborundum chips. The tubes were placed on an electric heating rack and the contents boiled gently until colourless. After cooling the total volume was made up to 10 mls. with distilled water. 1 ml. aliquots of the diluted sample were transferred to test-tubes containing 2 mls. 0.5N. sodium hydroxide (NaOH) and 1 ml. Nessler reagent. The optical density of the resultant solution was measured at 410 mμ using a Unicam S.P 500 spectrophotometer. Dilutions of a standard solution of ammonium sulphate containing 112 ppm. of nitrogen were read on each occasion and a standard curve constructed.

(ii) Trichloracetic acid (TCA) soluble and TCA insoluble nitrogen.

TCA soluble and insoluble nitrogen determinations were made by grinding batches of five segments in a hand manipulated ground glass homogeniser with 2 mls. 10% TCA and centrifuged at 2,500 g. for ten mins. The pellet was resuspended in a further 1 ml. 10% TCA, recentrifuged and the supernatant added to the first. The supernatants were evaporated to dryness and the nitrogen contents of the residues and of the pellets from the centrifugation were estimated by the Kjeldahl/Nessler procedure as above. TCA insoluble nitrogen may be considered as protein nitrogen and the weight of protein estimated by multiplying the weight of protein nitrogen by the statutory figure of 6.25.

In Series 1 total nitrogen was estimated separately from TCA soluble and insoluble nitrogen. But in Series 2 the total nitrogen values were found by summating the soluble and insoluble values for each segment.

d) Determination of chlorophyll content.

The chlorophyll content was determined by extracting the chlorophyll from batches of ten segments using the method of Arnon (1949). The segments were ground in a glass homogeniser with a total of 3 mls. 80% acetone and the suspension centrifuged at 2,500 g. for 10 mins. The supernatant was poured

off and kept and the pellet resuspended and recentrifuged with a further 1 ml. of 80% acetone. The supernatants were bulked and their optical densities read at 645 and 663 mμ on a Unicam S.P 500 spectrophotometer. The chlorophyll content was estimated using the equations of Mackinney (1941). These gave the amounts of chlorophyll a, chlorophyll b, and, by addition, total chlorophyll.

e) Estimation of nucleic acid content

Estimations of nucleic acid content were made in Series 2 only.

(i) Estimation of ribonucleic acid (RNA)

The basic extraction method was that of Schneider, Hogeboom and Ross (1950) the method being adapted as recommended by Ingle (1962). Batches of twenty segments were used and these were homogenised in the cold (2 to 4°C. in a ground glass homogeniser with 5% perchloric acid (PCA). The homogenates were centrifuged at 2,500 g. for 10 mins. in a refrigerated centrifuge at 0°C. The pellet was successively resuspended and recentrifuged with 5% PCA (twice), 80% ethanol, 100% ethanol, ethanol:ether:chloroform (2:1:1) (twice) and ethanol: ether (3:1). Before centrifugation with ethanol:ether the suspension was heated to 75°C. in a water bath for 5 mins. The pellet was then dried and then the RNA extracted by alkaline hydrolysis with 0.3N. potassium hydroxide at 37°C. for one hour. The extract was acidified with PCA and placed in the cold for 10 mins. It was then recentrifuged and the supernatant decanted. The supernatant was brought to pH 7-8 with potassium hydroxide (KOH) and applied to Dowex 1 x 8 ion exchange resin in the chloride form. The resin was carried in conical centrifuge tubes and the operation was performed in the 'bulk' manner. After application of the supernatant, the resin was washed with 0.01 N sodium chloride (NaCl) and eluted with N.HCl containing 2.3% NaCl according to the method of Smillie and Krotkov (1959). Each sample was eluted twice and, in the standard procedure, the eluates were bulked for RNA estimation. The optical densities of the eluates were read around 260 mμ on a Unicam S.P 800 recording spectrophotometer. The peaks at 260 mμ were quite sharp indicating a reasonable purity of RNA. The absolute quantities of RNA were calculated from an Ep value of 9,000 (Heyes 1966, personal communication).

(ii) Estimation of Desoxyribonucleic acid (DNA)

Chemical extraction of DNA was found to be unsatisfactory particularly when mature tissue was used. Similar results were reported by Kupila, Bryan and

Stern (1962). The DNA content of individual nuclei in selected segments was therefore measured by the Feulgen photometric method of Walker and Richards (1959). No attempt was made to relate the values found to absolute quantities of DNA. Longitudinal sections 15μ thick were cut on a microtome from wax embedded segments, and stained by the Feulgen technique after 6 mins. hydrolysis in N.HCl. The density of the staining of individual nuclei was measured using a Barr and Stroud integrating microdensitometer. The segments used were S1 at days 3, 6 and 10 and S4 at days 3, 6 and 10; 50 nuclei selected at random were measured in each segment.

7) The determinations of the metabolic characteristics of the leaf.

a) Respiration.

Respiration was measured in a Warburg respirometer using the modifications described by Umbreit, Burris and Stauffer (1959). Batches of ten segments were placed on a ring of filter paper in the annular space of the Warburg flasks. The paper was moistened with 1 ml. distilled water. The standard technique of shaking segments with a free liquid could not be used since it was found that some of the segments, being curled up into a cylindrical form, trapped air within them, and this air was not disturbed by shaking. Determinations involving floating segments gave highly variable and irregular results. With the method adopted normal shaking was applied for 2 hours and 20 mins. and manometer readings were taken every half hour after an initial 20 min. period of equilibration. With the modified technique constant rates of respiration were obtained.

The above technique was used in Series 1, in Series 2 the measurements of respiration were combined with those of photosynthetic rate.

b) Photosynthesis and Respiration (Series 2)

Photosynthetic activity was only measured in the segments of Series 2 and on this occasion respiration rate was measured simultaneously. The equipment used was a Braun's Model V58 Warburg bath equipped with a lighting system consisting of 14 X 40 watt, 240 volt bulbs whose light output was controlled by a rheostat. The bulbs were mounted below the Perspex bath and were kept at maximum intensity throughout the experimental period. It was found necessary to install a copper cooling coil to maintain a constant temperature in the water

bath. The light intensity at the surface of the segments was 500 ft. candles.

The first method tried for the measurement of photosynthesis was that of using alternate light and dark phases with a period of equilibration after each phase. However, with the small amount of material available, a phase length of at least one hour was necessary. Thus the technique demanded two light periods of one hour and a dark period of one hour. With three equilibration periods of twenty minutes, one between each phase, the total experimental time was four hours. Early experiments showed that the material could not stand up to such extended times and an alternative method was adopted.

Since it was found that the material grew in a very reproducible manner and that different batches of segments had very similar quantitative characteristics it was considered justifiable to perform the light and dark phase measurements on separate batches of segments at the same time. The segments for which respiration rate alone was being measured were placed in flasks containing a ring of filter paper moistened with 1 ml. distilled water and with 0.2 ml. 0.5N.KOH in the centre well. These flasks were surrounded with black polythene. The segments for which the combined rates of photosynthesis and respiration were being measured were supplied with 1 ml. bicarbonate buffer as recommended by Pratt (1943). This buffer contains 0.035 M potassium carbonate and 0.65 M sodium carbonate. Pratt found that with this buffer, the depressive effects of the sodium ions and the accelerating effects of the potassium ions were balanced and an even rate of photosynthesis could be maintained. The centre wells of these flasks were also supplied with 0.2 ml. 5N. KOH.

All the flasks were placed in the water bath at 25°C. with the lights switched on. 20 mins. were allowed for equilibration and readings were then taken over a period of two hours at half-hourly intervals. With this technique, the changes in the manometer readings were found to be more or less constant throughout the experimental period.

The details of manipulation of the Warburg apparatus, the calculation of flask constants and of the rates of photosynthesis and respiration were as described by Umbreit, Burris and Stauffer (1959).

Fig.4

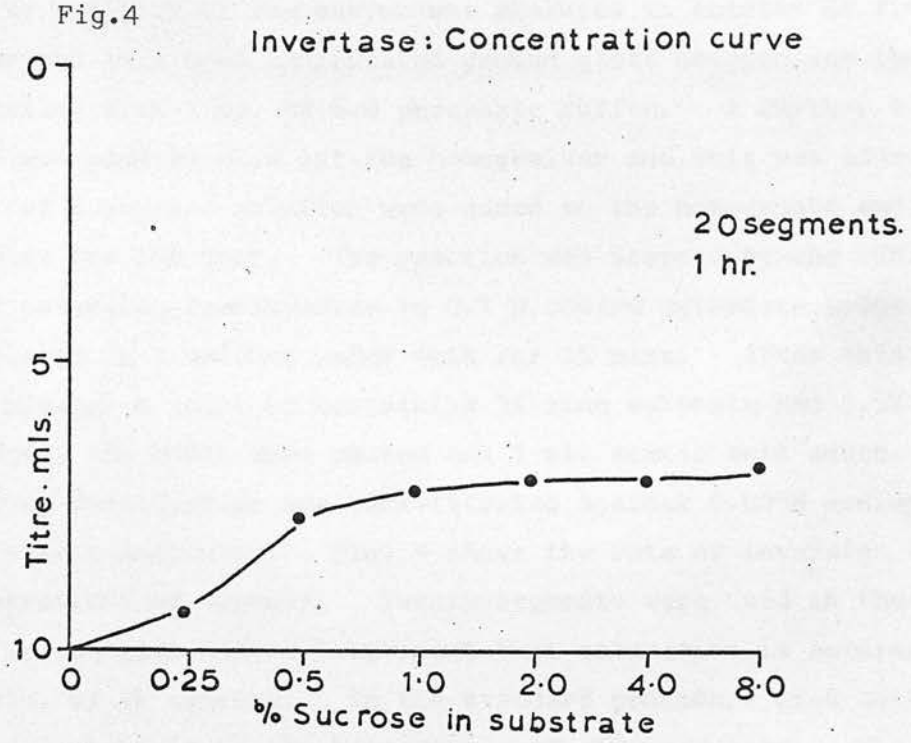
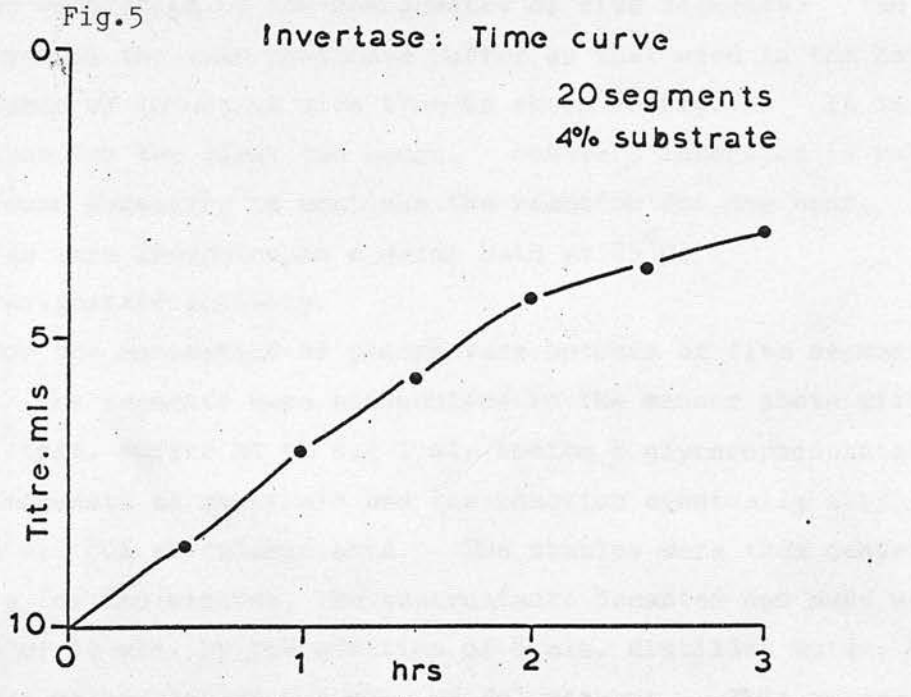

Invertase: Concentration curve
20 segments.
1 hr.
0
5
10
Titre. mls.
0
0·25
0·5
1·0
2·0
4·0
8·0
% Sucrose in substrate

Fig.5


Invertase: Time curve
20 segments
4% substrate
0
5
10
Titre. mls
0
1
hrs
2
3

c) Invertase activity.

The technique for the estimation of invertase activity was based on that of Robinson and Brown (1952, 1954). The amount of reducing sugar produced was estimated by the method of Hagedorn and Jensen (1923).

The activity of the enzyme was measured on batches of five segments, these were ground in a hand manipulated ground glass homogeniser immediately after preparation with 1 ml. pH 6.0 phosphate buffer. A further 1 ml. of the same buffer was used to wash out the homogeniser and this was added to the homogenate. 2 mls. of a sucrose solution were added to the homogenate and the mixture was incubated for one hour. The reaction was stopped by the addition of 20 mls. 0.005M potassium ferricyanide in 0.1 M sodium carbonate solution and the tubes were placed in a boiling water bath for 15 mins. After this they were cooled and 5 mls. of a solution containing 5% zinc sulphate and 2.5% potassium iodide was added, the tubes were shaken and 1 ml. acetic acid added. Finally the remaining ferricyanide was back-titrated against 0.005M sodium thiosulphate using starch indicator. Fig. 4 shows the rate of inversion with changing concentrations of sucrose. Twenty segments were used in the preparation of these homogenates and it is evident that saturation is secured by the addition of 2 mls. of 2% sucrose. In the standard procedure used here 2 mls. of 4% sucrose were added to the homogenates of five segments. The sucrose was dissolved in the same phosphate buffer as that used in the homogenisations. The course of inversion with time is shown in Fig. 5. It is evident that this is linear for the first two hours. However, inversion is rapid and it was only found necessary to continue the reaction for one hour. The reaction mixtures were incubated in a water bath at 25°C.

d) Phosphatase activity.

For the estimation of phosphatase batches of five segments were again used. The segments were homogenised in the manner above with a total of 2 mls. tris. buffer at pH 6.2 1 ml. sodium β glycerophosphate was added to the homogenate as substrate and the reaction eventually stopped by the addition of 0.8 ml. 60% perchloric acid. The samples were then centrifuged at 2,500 g for ten minutes, the supernatants decanted and made up to a final volume of 10 mls. by the addition of 5 mls. distilled water, 0.4 mls. 8.3% ammonium molybdate and 0.8 mls. amidol reagent. This reagent was freshly prepared on each occasion and consisted of 8 mls. distilled water in which 2 gms. sodium metabisulphite were dissolved and to this was added 2 mls. of distilled water containing 0.1 gms. amidol. Full colour was developed after

Fig.6

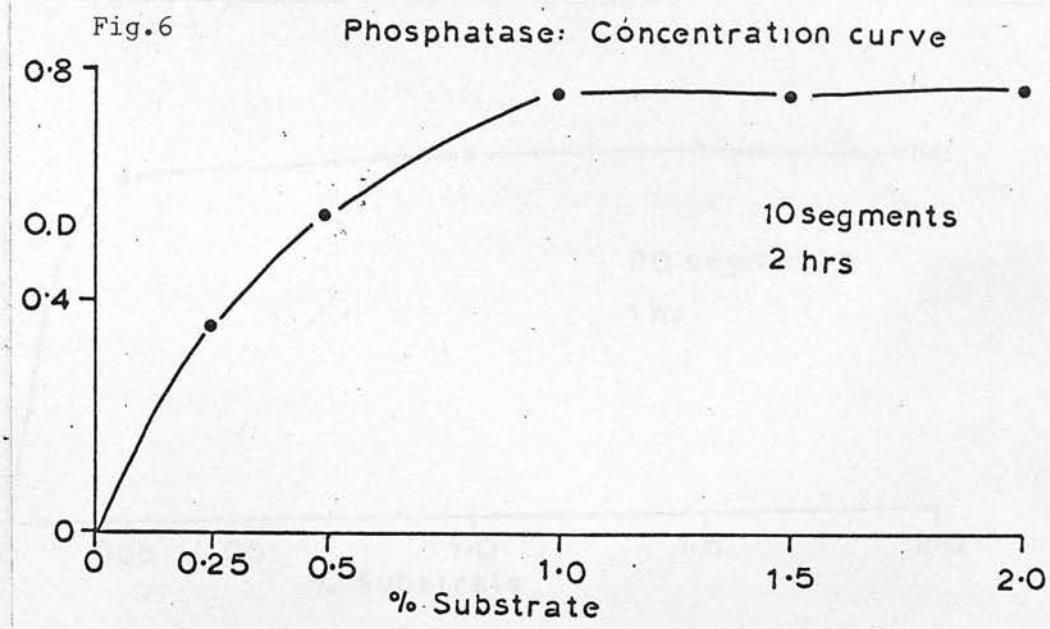

Phosphatase: Concentration curve
0·8
O.D
0·4
0
10 segments
2 hrs
0
0·25
0·5
1·0
1·5
2·0
% Substrate

Fig.7

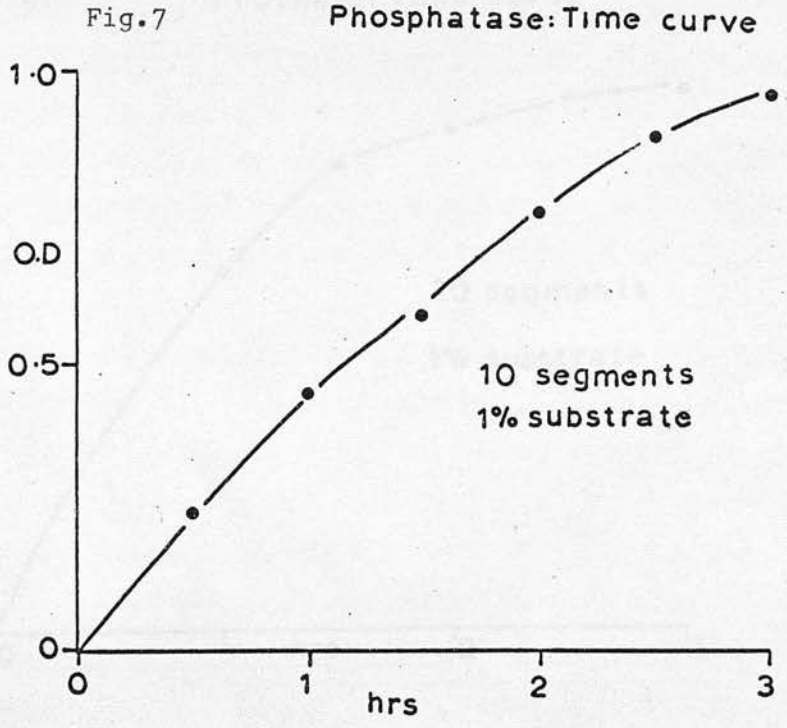

Phosphatase: Time curve
1·0
O.D
0·5
0
10 segments
1% substrate
0
1
2
3
hrs

Fig.8 Protease: Concentration curve

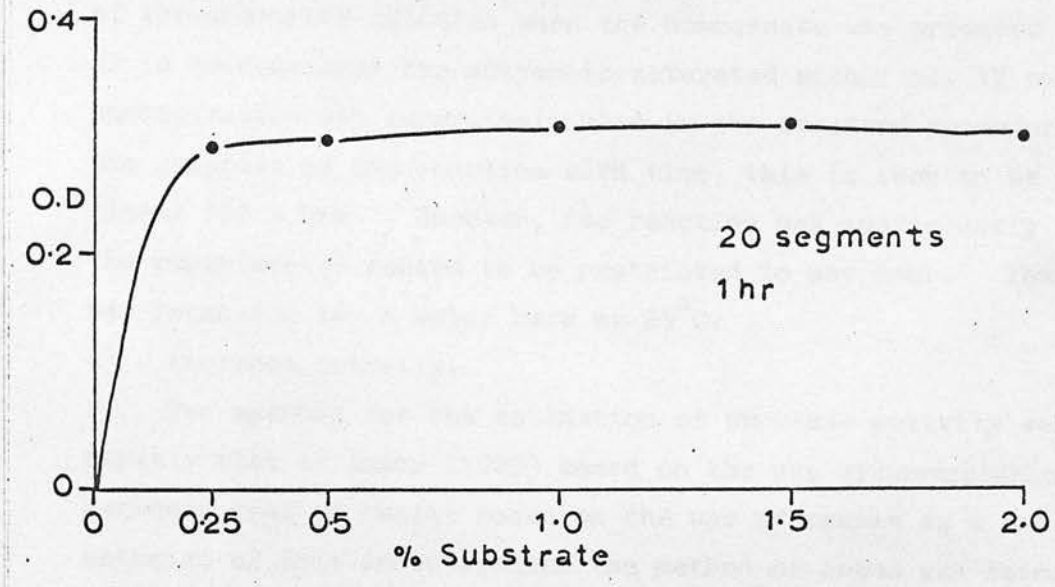
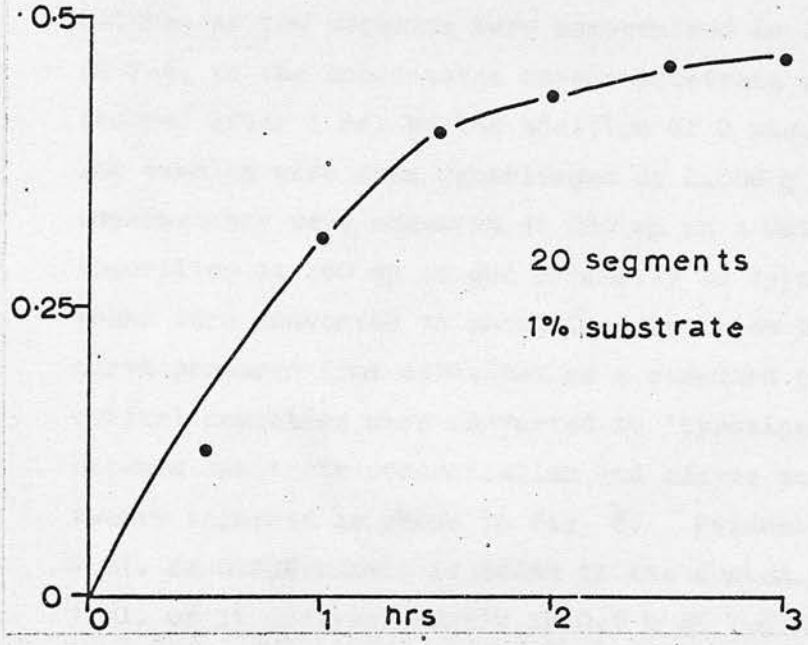


Fig.9 Protease: Time curve



20 mins. and the optical densities of the solutions were read at 725 mμ on a Unicam S.P 500 spectrophotometer. The optical densities were converted to μgms of inorganic phosphate from a calibration curve made up by using dilutions of a standard solution of potassium dihydrogen phosphate. This method of estimation of inorganic phosphate is basically that of Allen (1940).

Fig. 6 shows the effect on reaction rate of changing the concentration of the substrate solution when the homogenate was prepared from ten segments. It is evident that the enzyme is saturated with 1 ml. 1% substrate and this concentration was accordingly used in the standard procedure. Fig. 7 shows the progress of the reaction with time, this is seen to be more or less linear for 3 hrs. However, the reaction was sufficiently vigorous to allow the experimental period to be restricted to one hour. The reaction mixture was incubated in a water bath at 25°C.

e) Protease activity.

Two methods for the estimation of protease activity were explored. Firstly that of Anson (1929) based on the use of haemoglobin as a substrate. Secondly that of Kunitz based on the use of casein as a substrate. With the material of this investigation the method of Anson was found to be unsuitable and had to be discarded. The method of Kunitz, as described by Laskowski (1955) proved to be satisfactory and was adopted with some modifications. Batches of ten segments were homogenised in 2mls. 0.1 M phosphate buffer at pH 7.6, to the homogenates casein substrate was added the reaction being stopped after 1 hr. by the addition of 2 mls. 10% trichloracetic acid. The samples were then centrifuged at 2,500 g and the optical densities of the supernatants were measured at 280 mμ on a Unicam S.P 500 spectrophotometer. Absorption at 280 mμ is due primarily to tyrosine and tryptophan and the values found were converted to absolute quantities by comparison with a calibration curve prepared from dilutions of a standard tyrosine solution. Thus the optical densities were converted to 'tyrosine equivalents'. The relation between substrate concentration and enzyme activity for homogenates from twenty segments is shown in Fig. 8. Evidently the enzyme is saturated when 1 ml. of 0.25% casein is added to the system. In the standard procedure 1 ml. of 1% dialysed casein in 0.1 M pH 7.6 phosphate buffer was added to the homogenates. The curve for reaction with time, Fig. 9, shows that the rate declines progressively after one and a half hours. In the method used digestion was continued for one hour only. The reaction mixtures were incubated at 25°C in a water bath.

Presentation of Results

In the next section, the results of the various determinations are given in graphical form; each value in the figures being the mean, usually of three determinations. The numerical values for the means, together with the tables of standard errors of the original separate determinations, are given in the Appendices. The quantitative data for Series 1 and 2 are presented first and then the metabolic data.

Results

a) Investigation of growth regions and meristematic activity.

i) Marking experiments.

Introduction

Before the main body of observations could be undertaken, it was clearly necessary to establish the characteristics of the leaf with respect to cell division and cell expansion. It was also desirable to determine how the incidence of these processes in the different regions of the leaf varied with time. Accordingly, a preliminary series of observations were undertaken to establish these basic data.

The investigation was conducted by placing marks at known intervals along the first leaves of seedlings of various ages. The leaves could, subsequently, be measured and the growth between the marks determined. De Ropp (1946) used a similar technique for measuring the growth regions of first leaves of rye seedlings, but this investigation was confined to recently-germinated seedlings with plumules 1.2 mm. long. De Ropp completely removed the coleoptiles of his seedlings before applying marks and stated that he found no depressive effect on growth from this treatment. This finding contrasts with those of the present work in which it was found that removal of, or indeed damage to, the coleoptile always had a depressive effect on the subsequent growth of the seedling.

Furthermore, the younger the seedlings were at the time of treatment, the more drastic were the effects.

Throughout the course of the investigation described below, attempts were made to keep the damage to the coleoptile and to the leaf itself to a minimum. The methods of marking tried in this respect are described under 'Methods'; the final and most successful one being that of cutting small 'windows' in the coleoptile through which marks could be placed on the leaf. Since these marks were at known distances from the apex and base of the leaf, the regions of leaf growth could be established and, by dissection at the marks, the cell increment in the different regions of the leaf for known periods of time could be found.

Results

Experiment I

Since de Ropp had found that the removal of the coleoptile from very young rye seedlings did not affect their growth, it was decided to use seedlings 24 hours after germination in the first experiment.

A sample of six seedlings was taken, coleoptiles were removed, and the first leaves were marked at 1 mm. intervals from the apex. The seedlings were then replanted. It was hoped that when the distances between the marks were subsequently measured the principal areas of growth of the leaf could be established. It was known that the meristem was basal but the extent of this meristem was unknown as was the extent of the region of cell expansion. The distances between the marks were, therefore, re-measured 24 and 48 hours after marking, i.e. 48 and 96 hours after germination.

It was found that at 48 hours, two of the seedlings had been so drastically affected by the treatment that their first leaves had curled up and useful measurements could not be made.

Measurements on the remaining four seedlings are recorded in Table I below.

TABLE I

Age	24 hours		48 hours		96 hours	
Segments	Distance between marks in mms.		Distance between marks in mms.		Distance between marks in mms.	
Apex (1)	a) 1.0	b) 1.0	a) 1.5	b) 1.0	a) 1.5	b) 1.0
(2)	1.0	1.0	2.0	3.0	2.0	3.0
Base (3)	1.5	1.5	3.0	3.0	13.0	19.0
Total	3.5	3.5	6.5	7.0	16.5	23.0
Apex (1)	c) 1.0	d) 1.0	c) 1.0	d) 1.0	c) 1.0	d) 1.0
(2)	1.0	1.0	1.5	1.5	1.5	1.5
(3)	1.0	1.0	2.0	2.0	2.0	2.0
(4)	1.0	1.0	2.5	2.5	3.0	3.0
Base (5)	1.0	1.0	4.0	2.5	28.0	14.0
Total	5.0	5.0	11.0	9.5	35.5	21.5

From Table I above it can be seen that two of the surviving seedlings a) and b) had first leaves 3.5 mms. long at 24 hrs. while the other two c) and d) had first leaves 5 mm. long. The rate of growth and also the final length of these seedlings was far less than that achieved by untreated seedlings. The mean length at 96 hours was normally 7.0 cms., whereas after marking the lengths achieved after 96 hours varied between 1.65 and 3.55 cms.

This variation was probably due to differing amounts of mechanical damage suffered by the seedlings during the removal of the coleoptile and the marking of the leaves. However, despite the limited growth, it was shown that it practically all occurred in the basal segment.

It was interesting that the removal of the coleoptile resulted in much earlier growth of the stem, which took over the function of supporting the plant. When the coleoptile was removed from older plants, these usually collapsed, demonstrating that the coleoptile was not only responsible for the protection of the shoot in its passage through the soil, but also for the early support of the shoot.

Experiment 2

From Experiment 1, it appeared that 24 hr. seedlings would not be satisfactory for this investigation. It was decided, therefore, to use rather older material which, it was hoped, would be better established and would be less drastically affected by mechanical damage. The seedlings would also have to be small enough for their shoots not to collapse on removal of the coleoptile. On this occasion, therefore, a sample of six seedlings 48 hrs. after germination was taken, coleoptiles removed and marks placed at appropriate intervals on the leaves. Experiment 1 had shown that the apical portion of the leaf expanded little, if at all, so the first mark was placed 5 mm. from the apex. On accurate measurement, three of the seedlings were found to be 12 mm. long and the other three 14 mm. long. It was, therefore, decided to place one mark 1 mm. from the base of the first leaf of all seedlings with subsequent marks at 2 mm. intervals up to the sub-apical mark mentioned above, thus the 14 mm. seedlings had one more mark than the 12 mm. seedlings.

The results of the experiment are shown in Table II below:

TABLE II

Age	48 hours			72 hours			96 hours		
Segments	Distance between marks in mm.			Distance between marks in mm.			Distance between marks in mm.		
Apex (1)	a) 5.0	b) 5.0	c) 5.0	a) 5.0	b) 5.0	c) 5.0	a) 5.0	b) 5.0	c) 5.0
(2)	2.0	2.0	2.0	2.0	2.5	2.0	2.0	2.5	2.0
(3)	2.0	2.0	2.0	3.5	4.5	4.0	3.5	4.5	4.0
(4)	2.0	2.0	2.0	5.0	6.0	6.0	6.0	7.0	8.0
Base (5)	1.0	1.0	1.0	4.0	3.0	4.0	17.0	15.0	14.0
Total	12.0	12.0	12.0	19.5	21.0	21.0	33.5	34.0	33.0
Apex (1)	d) 5.0	e) 5.0	f) 5.0	d) 5.0	e) 5.0	f) 5.0	d) 5.0	e) 5.0	f) 5.0
(2)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
(3)	2.0	2.0	2.0	3.0	2.5	3.0	3.0	2.5	3.0
(4)	2.0	2.0	2.0	5.0	5.0	5.0	5.0	5.0	5.0
(5)	2.0	2.0	2.0	5.0	7.0	5.0	5.0	9.0	8.0
Base (6)	1.0	1.0	1.0	4.0	3.0	3.0	15.0	11.0	10.0
Total	14.0	14.0	14.0	24.0	24.5	23.0	35.0	34.5	33.0
Controls	12-14 mm.			30-34 mm.			65-70 mm.		

Table II above shows the growth of six individual seedlings marked as described above and replanted 48 hrs. after germination. Three of the seedlings: a, b, and c were 12 mm. long at 48 hrs. and the other three: d, e, and f were 14 mm. long. The distances between the marks were remeasured at 72 and 96 hrs. As can be seen from the table, there was no increase in length in the apical 5 mm. and only on one occasion b) was there any increase in segment 2. Segment 3 showed a rather greater increase in leaves a, b, and c than in d, e, and f. The basal showed similar increases in length in all leaves, though there was some variation. Segment 4 of a, b, and c was also very similar to segment 5 of d, e, and f.

The overall lengths achieved by the six leaves were very similar, though only about half that of the controls which were transplanted at the same time.

From 48 to 72 hrs. the increase in length of the basal 3 mm. (i.e. the basal two segments) was fairly even, demonstrating that it was not only the basal 1 mm. that was responsible for growth. The increase in length of the basal 3 mm. of the leaves from 48 to 72 hrs. and from 72 to 96 hrs. was very similar. This suggests that the meristem was maintaining the same level of activity between 48 and 96 hrs. Although the leaves showed little mechanical damage, growth was greatly depressed and it was concluded that this was mainly due to the removal of the coleoptile.

Experiment 3

Since it appeared from Experiment 2 that the removal of the coleoptile was responsible for most of the depressive effect on growth, the coleoptiles were not removed in this experiment. Instead, small slits were cut in the positions where marks were required and the leaf exposed. So far only increases in length of regions of the leaf have been measured: these increases must, however, be the result of two processes, cell division and cell expansion. The next experiments were designed to determine the number of cells in the meristematic area, the constancy of this number with increasing age and the rate of cell number increment for the leaf as a whole. It was also of interest to discover if the cell number of the non-expanding region remained constant. Since 48 hr. seedlings were not so drastically affected by marking and were easier to handle than the 24 hr. ones, they were again used.

A sample of fifteen seedlings 48 hrs. after germination and 13 mm. in length was taken: five had their coleoptiles carefully slit and a mark placed on the leaf 5 mm. from the base. This left a segment of 8 mm. between the mark and the apex. Five were transplanted as controls while the remaining five had their first leaves removed from the coleoptiles and cut into two segments the basal one being 5 mm. and the apical one 8 mm. These segments were placed in 5% chromic acid for maceration before cell counting as described on page 28.

At 72 hrs. the lengths of the growth controls and the marked leaves were measured and the marked leaves were cut in two at the position of the marks. Since it was required to determine whether the number of cells in the meristem remained constant or not between 48 and 72 hrs., it was necessary that the segments representing the meristem at the two times should be of the same length. Therefore, the basal 5 mm. was cut off the lower segment of the 72 hr. marked leaves. This meant that the leaf was divided into three segments basal, intermediate and apical. These segments were placed in chromic acid for cell counting. The average lengths of the segments and their cell numbers are shown in Table III below:

TABLE III

Segment	Average length of segment in mm.		Average cell number per segment	
	48 hrs.	72 hrs.	48 hrs.	72 hrs.
Base	5.0	5.0	159,375	215,375
Inter.	-	10.2	-	116,800
Apex	8.0	10.0	91,250	77,812
Total	13.0	25.2	250,625	409,987
Control	13.0	31.3		
Increment in cell number per leaf. 159,362			Cell increment per leaf per hr. 6,640	

From Table III above it can be seen that there is little effect on the growth of the first leaf as the result of transplanting. The total lengths of the marked leaves was very similar to those recorded in Experiment 2 showing that the effect of slitting the coleoptile was no less depressive on growth than its removal. There was a considerable increase in the cell number of the basal 5 mm. from 48 to 72 hrs., this represents an increase in the size of the meristem. The upper part of this 5 mm. which contained expanding cells at 48 hrs. presumably contains smaller dividing cells at 72 hrs.

The apical 8 mm. only increased in length by 2 mm. and this was probably due solely to cell expansion; indeed there was an apparent decrease in cell number in this region, but this was thought to be due to experimental error. The cells of the apical segment at 72 hrs. had reached their full size and this can be considered as the maturing region. It was thought unlikely that there was much meristematic activity in the intermediate segment so this was taken to be the zone of cell expansion.

If the basal 5 mm. at 48 hrs. is wholly responsible for the increase in cell number of the leaf at 72 hrs. then the average length of the mitotic cycle for the cells of this basal segment was 24 hrs., since the increment in cell number from 48 to 72 hrs. was the same as the cell number of the basal segment at 48 hrs.

Experiment 4

Several important points had been raised in Experiment 3 and it was decided to repeat the experiment in order to check their validity.

Experiment 4 was exactly the same as Experiment 3, with the exception that the seedlings used were 11 mm. in length and no controls were transplanted. The results are shown in Table IV below:

TABLE IV

Average length of segment in mm.			Average cell number per segment	
Segment	48 hrs.	72 hrs.	48 hrs.	72 hrs.
Base	5.0	5.0	179,890	217,366
Inter.	-	10.7	-	114,088
Apex	6.0	6.5	45,156	47,943
Total	11.0	22.2	226,046	379,397
Increment in cell number per leaf 153,351			Cell increment per leaf per hr. 6,389	

The results confirm those of Experiment 3, in showing that:

a) The cell number in the basal meristem increases from 48 to 72 hrs., in this case by ~~38~~,000 and previously by ~~66~~,000.

b) The apical region increases little in length and not at all in cell number, the apparent loss in cell number found in Experiment 3 was not repeated.

c) The rate of cell increment about 6,500 per hour was the same in both experiments as was the average duration of the mitotic cycle for the basal 5 mm.

Experiment 5

The results recorded above, while showing that the effect of damaging or removing the coleoptile is to depress growth, do not show whether this is due to a reduction in cell division, cell expansion, or both.

An experiment was, therefore, designed to investigate these possibilities. A sample of six 48 hr. seedlings 13 mm. in length was used and the apical 6 mm. of the coleoptile removed from three of them. The remaining three were used as controls and all six were replanted. After a further 24 hrs. the first leaves were removed and placed in chromic acid for determination of cell number. The final lengths and cell numbers of the leaves are recorded in Table V below:

TABLE V

Lengths of leaves at 48 hrs. 13 mm.	
Lengths of leaves at 72 hrs.	
With whole coleoptiles, in mm. 35, 33, 33.	With half coleoptiles, in mm. 24, 25, 25.
Cell no. per leaf 422,500	Cell no. per leaf 331,460
Difference 91,040	

The data above show that a reduction in length of 8 mm. per leaf was accompanied by a reduction in cell number of 91,000 when half the coleoptile was removed. If it is assumed that the basal 5 mm. in each case had the same cell number, and this is supported by later data, then the reduction in cell number may be considered as occurring in the intermediate and the maturing zones. The average cell number per mm. in these zones is around 10,000, thus 90,000 cells is equivalent to 9 mm. of tissue. This approximates closely to the difference in length between the two sets of leaves in the table above. Thus it was concluded that the effect of removing or damaging the coleoptile was principally on cell division.

In Tables III and IV it was seen that the meristem was confined, principally, to the basal 5 mm. of the leaf, also the average length of the mitotic cycle for the basal 5 mm. of the 48 hr. leaf was shown to be 24 hrs. It was not known if this rate was uniform throughout the basal 5 mm. and to investigate this it was necessary to place marks closer than 5 mm. from the base of the leaf.

Experiment 6

A sample of twelve seedlings 48 hrs. after germination and with leaves 11 mm. in length was taken. Small slits were made in the coleoptiles of six of the seedlings and marks placed on the leaves at 2 mm. and 4 mm. from the base. The other six were cut into three segments, a basal segment 2 mm. long, an intermediate segment 2 mm. long and an apical one 7 mm. long. These segments were placed in chromic acid for determination of cell number. The marked seedlings were replanted and allowed to grow for a further 24 hrs. after which their first leaves were also cut into segments at the marks, the lengths of the segments measured and placed in chromic acid. The lengths and cell numbers of the segments are shown in Table VI below:

TABLE VI

Average length of segment in mm.			Average cell number per segment	
Segment	48 hrs.	72 hrs.	48 hrs.	72 hrs.
Base	2.0	6.1	89,739	244,947
Inter.	2.0	6.1	75,051	93,333
Apex	7.0	9.8	8,749	7,332
Total	11.0	22.0	226,035	410,155
Increment in cell no. per leaf 184,120			Cell increment per leaf per hr. 7,672	

The data above clearly show that while the two 2 mm. segments both attained a length of 6.1 mm. at 72 hrs., it was the basal 2 mm. that was principally responsible for the increase in the cell number of the leaf.

In this case, 86% of the cell increment occurred in the original basal 2 mm. segment. If the number of meristematic cells in the basal 2 mm. is assumed to be the same at 72 as at 48 hrs., this cell increment means that the mitotic cycle of the cells in the basal segment was being completed in an average of about 14 hrs. The next 2 mm. contributed only 20,000 cells, so a considerable amount of cell expansion must have taken place here to account for the threefold increase in length. Cell expansion also occurred in the apical segment but clearly from the data no cell division took place here, indeed there was an apparent loss of cells from this region.

The effect of placing marks nearer to the base of the leaf was to depress growth even further than in previous experiments.

Experiment 7

It was considered necessary to repeat the previous experiment and a refinement of the marking technique was introduced to try and lessen the depression of growth. This was to cut a small 'window' in the coleoptile in the position where a mark was required. These 'windows' were about 0.5 mm. square. Further, it was decided to assume that the apical segment would grow to the same length as in Experiment 6 and, thus, remove the necessity for a second mark.

A sample of twelve seedlings 48 hrs. after germination and 12 mm. in height was taken and one mark was placed 2 mm. from the base on six of the seedlings, the remaining six being cut into segments, as before, of 2 mm., 2 mm., and 8 mm. for cell number determinations. At 72 hrs. the apical 10 mm. was cut off and the rest of the leaf cut into two at the mark. Thus, three segments were available the length of the latter two being based on the results of Experiment 6.

The lengths of the segments and their cell numbers are shown in Table VII below:

TABLE VII

Average length of segment in mm.			Average cell number per segment	
Segments	48 hrs.	72 hrs.	48 hrs.	72 hrs.
Base	2.0	5.3	103,433	284,375
Inter.	2.0	7.2	67,000	90,937
Apex	8.0	10.0	70,343	86,250
Total	12.0	22.5	240,776	461,562
Increment in cell no. per leaf 220,786			Cell increment per leaf per hr. 9,199	

The data of Table VII support in general those of Table VI. The overall increment in length was smaller than that hoped for but there was an increase in cell number per leaf of 36,000 over the leaves of Experiment 6, this increase being entirely in the basal segment. However, since this segment had a greater number of cells at 48 hrs. the average duration of the mitotic cycle was very similar. The basal 2 mm. was responsible for 82% of the cell increment of the leaf on this occasion.

Having established the pattern of growth and cell division in the first leaf of wheat between 48 and 72 hrs., the study was extended to the period between 72 and 96 hrs.

Experiment 8

A sample of six seedlings 72 hrs. after germination was used and marks were placed on the leaves at 5 mm. intervals. As the apical centimetre had extended beyond the tip of the coleoptile by this time, it was only necessary to cut four 'windows'.

The seedlings were replanted for a further 24 hrs. and then the first leaves were taken and cut into segments at the marks. The segments were measured and placed in chromic acid for determination of cell number. As in Experiments 3 and 4, the basal 5 mm. was cut off the lowest segment in order to compare the number of cells in the meristematic regions at the two times. The segments were called 1a and 1b. The results of this experiment are shown in Table VIII below:

TABLE VIII

	Average length of segment in mm.		Average cell number per segment	
	72 hrs.	96 hrs.	72 hrs.	96 hrs.
Base (1)	5.0	1a) 5.0 1b) 16.0 1) 21.0	210,312	1a) 260,208 1b) 173,271 (1) 433,479
(2)	5.0	9.7	86,562	112,812
(3)	5.0	5.25	41,265	46,250
(4)	5.0	5.0	38,833	40,937
(5)	5.0	5.0	33,271	39,315
(6)	5.0	5.0	32,167	38,000
Apex (7)	5.0	5.0	24,165	24,594
Total	35.0	55.95	466,575	735,387
Cell increment per leaf 268,912			Cell increment per leaf per hr. 11,200	

The data above show an increase in cell number in the basal 5 mm. from 72 to 96 hrs., and the rate of cell increment per leaf per hr. found above is greater than that found in the previous experiments. Thus the size of the meristematic area and the rate of cell increment of the leaf increased from 48 to 72 to 96 hrs. The duration of the mitotic cycle, assuming as in Experiments 3 and 4 that all the cells in the basal 5 mm. are dividing, was again about 24 hrs. The table above shows that the basal segment increased in cell number by 100% and in length by 300% from 72 to 96 hrs. whereas segment 2 increased in cell number by 50% and in length by 100%.

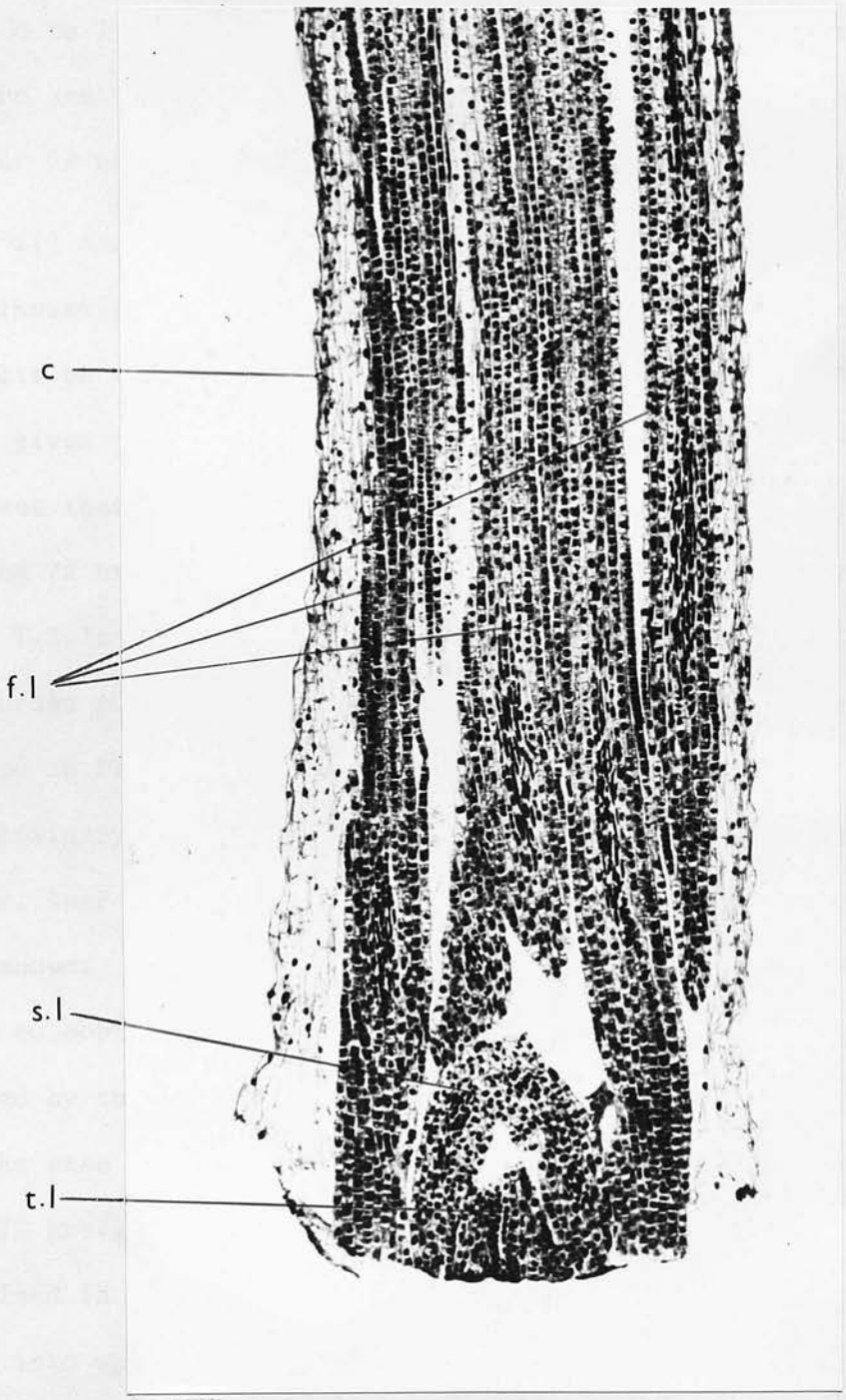


Plate 2

The basal region of a wheat shoot 48 hours after germination showing coleoptile (c) first leaf (f.l.) second leaf primordium (s.l.) third leaf primordium (t.l.) x 90.

Segment 3 increased only slightly in both cell number and length. Segments 4 to 7 did not increase in length and the increases in cell number found here are thought to be due to experimental error. The cell numbers quoted for 72 hrs. above are the mean values from Appendix I.

ii) Anatomy of the Leaf.

An investigation of the anatomy of the leaf was undertaken to clarify the results on the growth of the system presented above. For reasons that are given in a later section, the anatomical investigation was made with leaves that were approximately 1.2 and 3.5 cm. long and had been grown for 48 and 72 hrs. respectively after germination. The investigation is based on T.S.'s and L.S.'s of these leaves the characteristics of which were recorded photographically and a selection of the photographs are reproduced in Plates 2 to 22.

Preliminary observations showed that differentiation is limited in the 48 hr. leaf and, accordingly, only one L.S. through the base of this leaf is shown. For the anatomical investigation, this leaf was not separated from the coleoptile and Plate 2 shows the basal region of the first leaf surrounded by the coleoptile with the second and third leaf primordia within the base of the first leaf.

At 72 hrs., the leaf is more extensively developed and the anatomy was examined in detail with both L.S.'s and T.S.'s throughout the seven segments into which the leaf is dissected for experimental purposes. Sections are only shown through the basal four segments since the anatomy of the three terminal segments is similar to that of the fourth. A series of L.S.'s and T.S.'s is shown through representative regions of each of the first four segments in Plates 3 to 22.

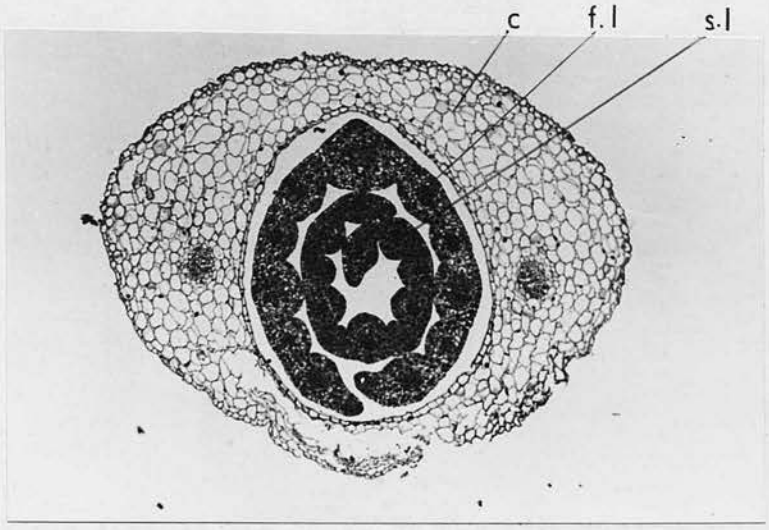


Plate 3 T.S. x 53

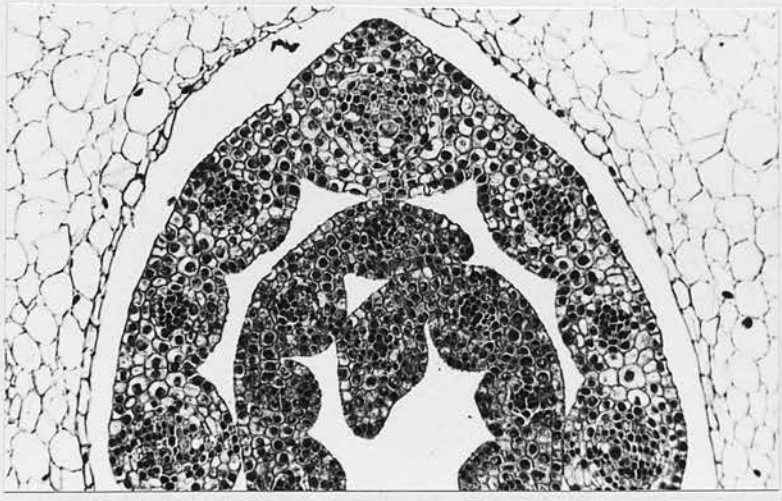


Plate 4 T.S. x 130

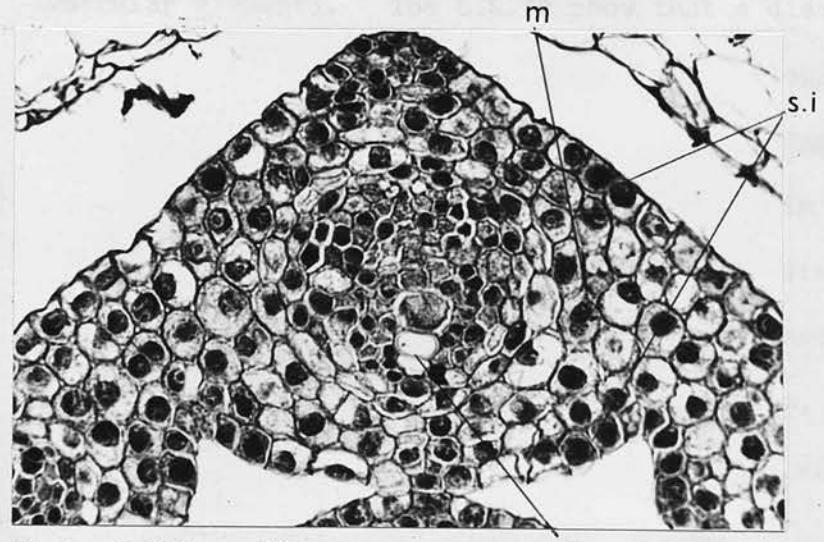


Plate 5 T.S. x 330

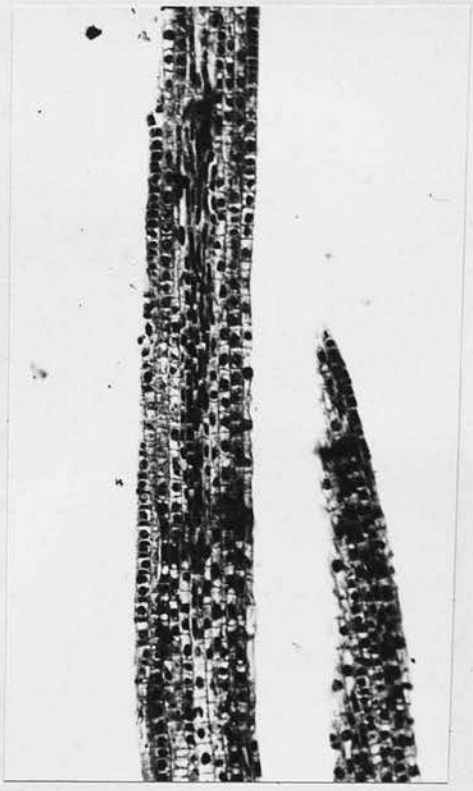


Plate 6 L.S. x 130

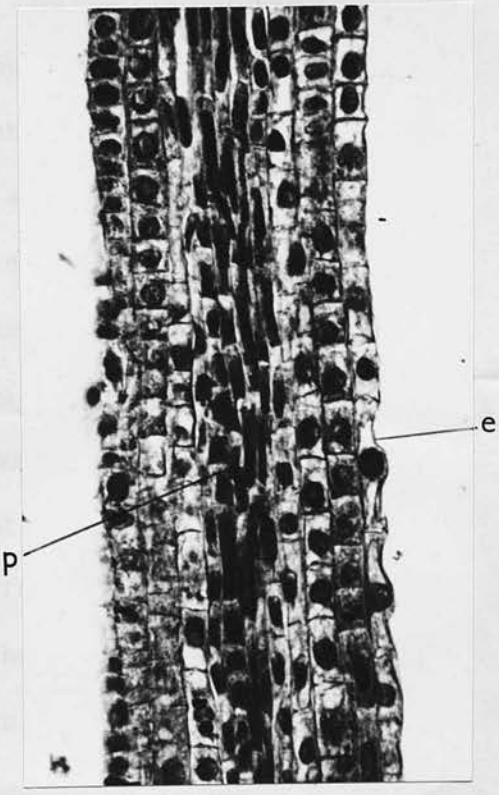


Plate 7 L.S. x 330

Plates 3-7 illustrate sections through S1 of Series 1 at 72 hrs.
c = coleoptile, f.l. = first leaf, s.l. = second leaf, m = mesophyll,
s.i. = stomatal initials, x = xylem, p = procambium, e = epidermis.

In the 48 hr. leaf, although differentiation is limited, the main histological regions may be distinguished. At the surface, an epidermis has been developed consisting of small, dense cells amongst which stomatal rows may be seen. Scattered throughout the leaf groups of cells elongated in the long axis of the leaf are present. These are the cells of the procambial strands, they have dense contents with large and prominent nuclei. Between the procambial strands, and between these and the epidermis, the tissues are those of the mesophyll and at this stage they consist of mostly non-vacuolated, isodiametric cells carrying large nuclei. It may be noted that, although the majority of the cells of the epidermis and the mesophyll are non-vacuolated, some of the cells of these tissues show indications of vacuolation. It is significant that this vacuolation does not begin at a certain level from the base in all cells but occurs sporadically in certain rows.

The details of the anatomy of the basal segment of the 72 hrs. leaf are shown in Plates 3 to 7. The T.S.'s show that the leaf is ridged on the inward-facing surface and that each ridge is associated with a group of vascular elements. The L.S.'s show that a distinctive epidermis is present on the surfaces and that some of the epidermal cells are vacuolated. The central vascular bundle consists of a procambial strand, the cells of which are elongated in the long axis with their contents heavily stained. In all but the central strand, there is no indication at this stage of differentiation into xylem and phloem. Between the procambial elements themselves and between these and the epidermis, the mesophyll tissue consists of densely-packed isodiametric cells with large nuclei.

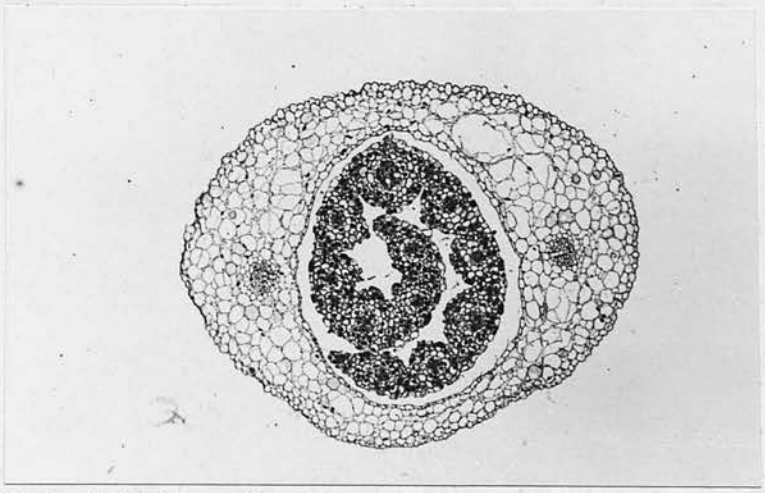


Plate 8 T.S. x 53

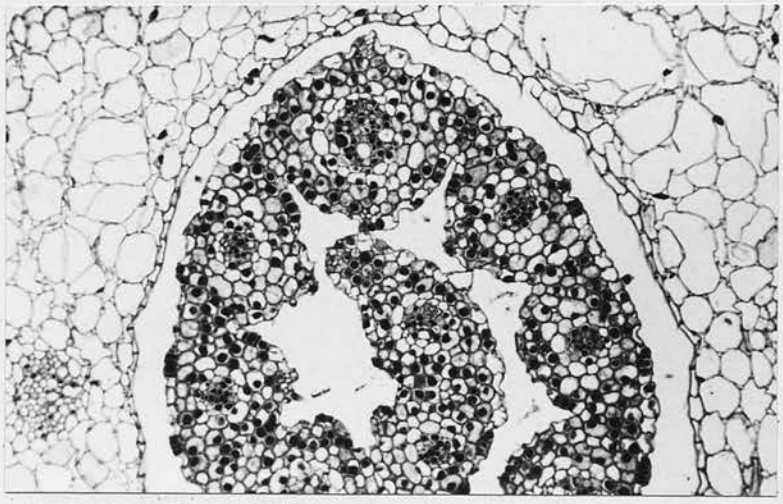


Plate 9 T.S. x 130

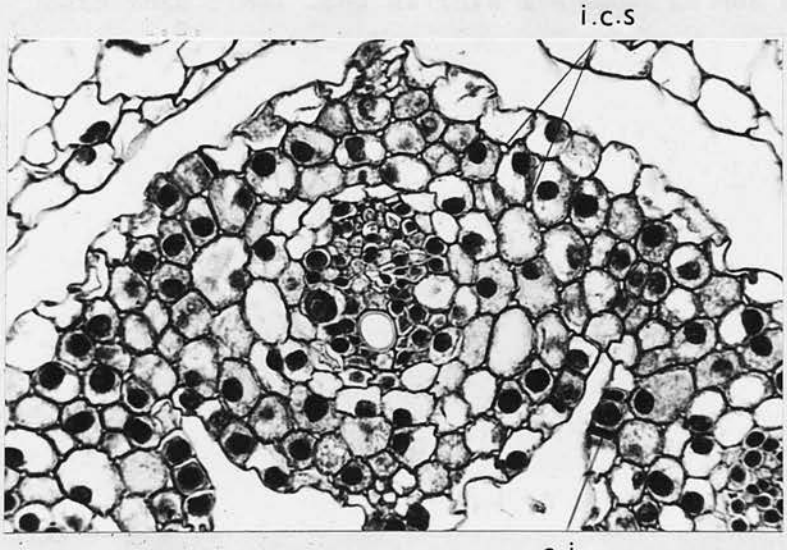


Plate 10 T.S. x 330

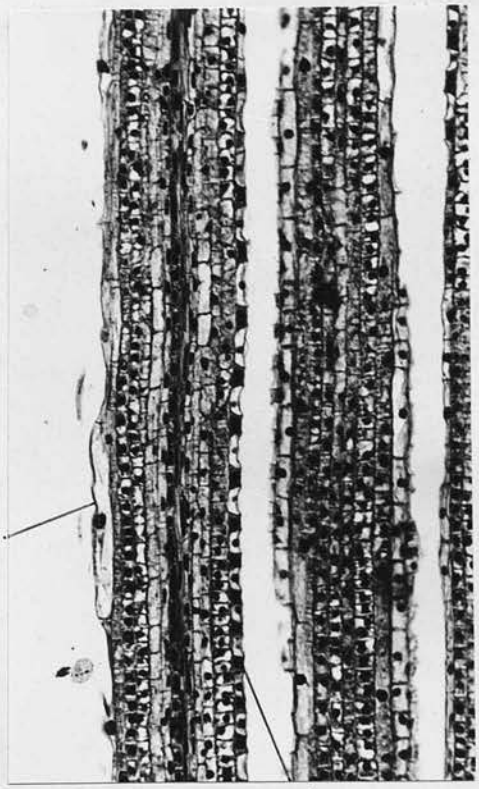


Plate 11
L.S. x 130

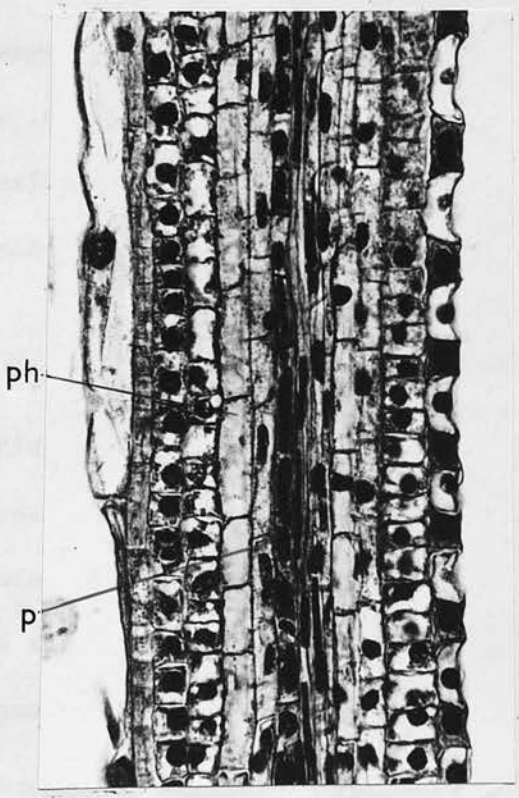


Plate 12 L.S. x 330

Plates 8-12 illustrate sections through S2 of Series 1 at 72 hrs.
i.c.s. = intercellular space, s.i. = stomatal initials, e = epidermis,
ph. = phloem, p = procambium.

Some of these mesophyll cells have, however, begun vacuolation. Both T.S.'s and L.S.'s show that except in the central axial region, intercellular spaces are either very small or absent. In the epidermis, stomatal initials are present, one row being developed on each side of each vascular bundle on both surfaces. Four rows of stomatal initials are, therefore, associated with each vascular bundle. As can be seen from Plate 5, the stomatal rows at this time are in the two-celled stage. The central vascular bundle differs from the others in that three well-defined xylem elements may be seen on its inner surface and these elements have thickened walls. In squash preparations, lignified spiral and annular bars of thickening have been identified in the walls of these elements. It is significant that in the mesophyll tissue surrounding the central bundle, the cells tend to be larger than elsewhere and intercellular spaces may also be seen.

The sections of the 72 hr. lear segments were all made with the coleoptile in position and this is shown in the low magnification photographs. In S1 the T.S.'s also cut through the second leaf; however since this is less than 5 mm. long at this stage, it is not seen in sections of S2 or later segments.

The anatomy of S2 is shown in Plates 8 to 12. Differentiation in S2 is more clearly defined than in S1 and the ridges on the inner surface of the leaf are more prominent. Epidermal tissue is sharply distinguished on both surfaces, as are the four rows of stomatal initials associated with each vascular bundle. The T.S's show that the stomatal initials are in the three-celled stage, there being a central guard mother cell with two flanking subsidiaries cells.

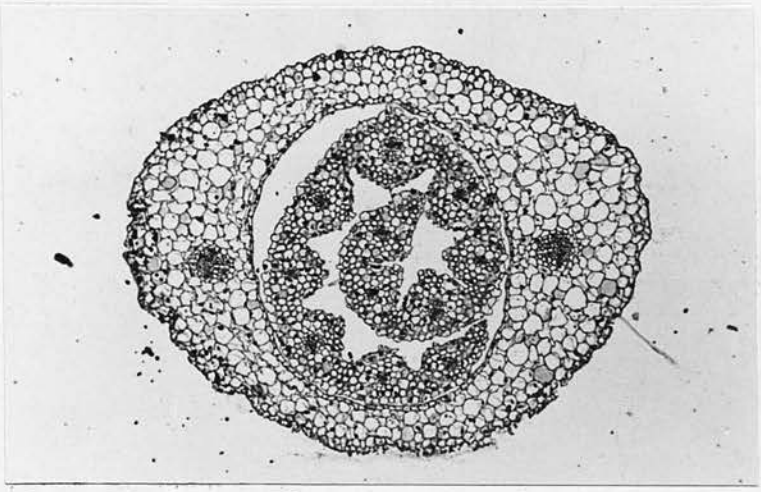


Plate 13 T.S. x 53

Plate 16 L.S. x 130

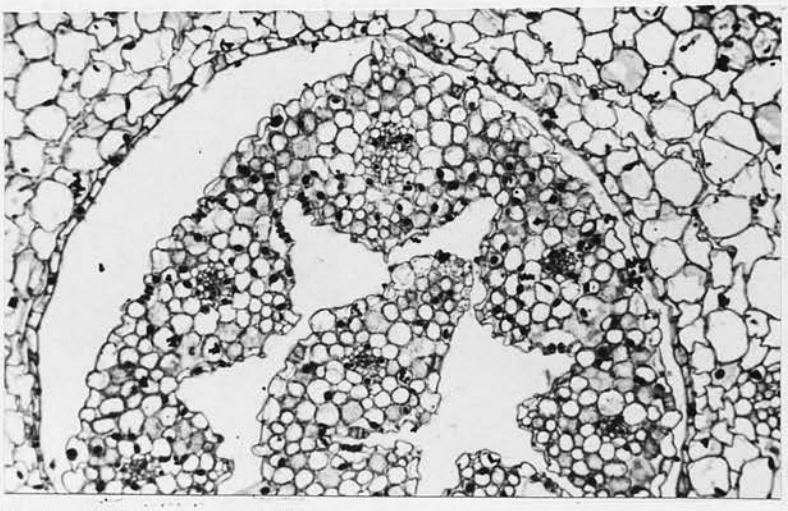


Plate 14 T.S. x 130

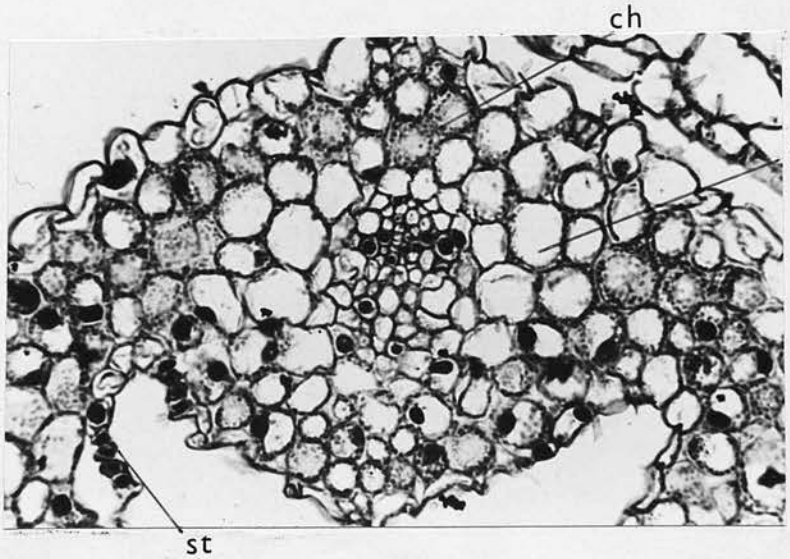


Plate 15 T.S. x 330

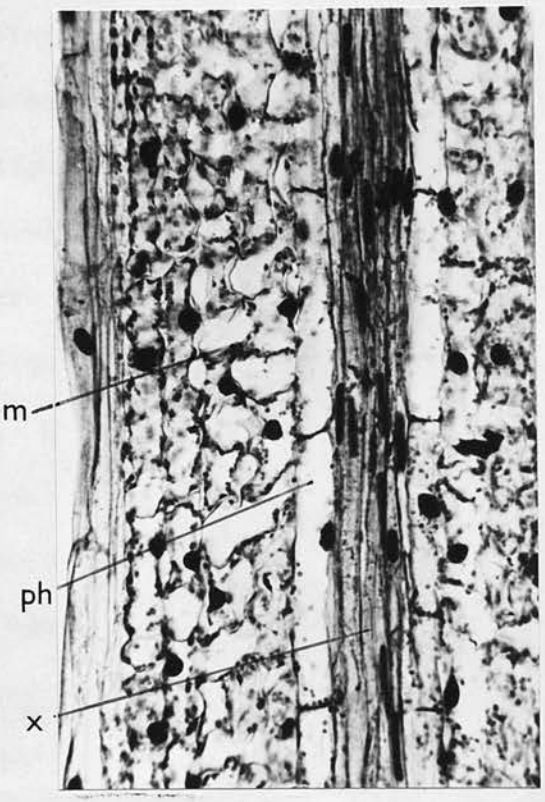


Plate 17 L.S. x 330

Plates 13-17 illustrate sections through S3 of Series 1 at 72 hrs.
ch. = chloroplasts, m = mesophyll, ph. = phloem, x = xylem, st. = stomata.

Each ridge, of which there are eleven, is associated with a vascular bundle and the L.S in Plate 12 shows that phloem cells have now been differentiated although in the flanking bundles the xylem still consists of elongate procambial elements with long and prominent nuclei. The mesophyll tissue is still uniform though there are now well-defined intercellular spaces. The nuclei in the majority of the mesophyll and epidermal cells are in peripheral positions as a result, no doubt, of the onset of vacuolation. The central vascular bundle again differs from the rest in the possession of sharply-defined xylem elements.

The anatomy of S3 is shown in Plates 13 to 17. The epidermal cells are fully developed and in the T.S.'s the stomatal complexes are seen to be mature, two guard cells being evident in each stoma and sub-stomatal cavities are present below the stomata. In the lateral vascular bundles, the phloem is pronounced and fully differentiated but in the xylem, although some tracheidal elements have been formed, many elongated cells with large nuclei typical of the procambial strand are still present. The mesophyll consists of large expanded, elongate cells with large intercellular spaces between them, the nuclei are prominent but occupy a peripheral position and the cells contain a large number of well-defined chloroplasts. It is significant that the cell walls are highly convoluted and this, in conjunction with the large intercellular spaces, facilitates gaseous exchange. In the central bundle xylem and phloem regions are distinct with one or two large vessels in the xylem.

The structure of the tissues in S4 is shown in Plates 18 to 22. The low-power T.S (Plate 18) shows that the leaf is still surrounded by the coleoptile.

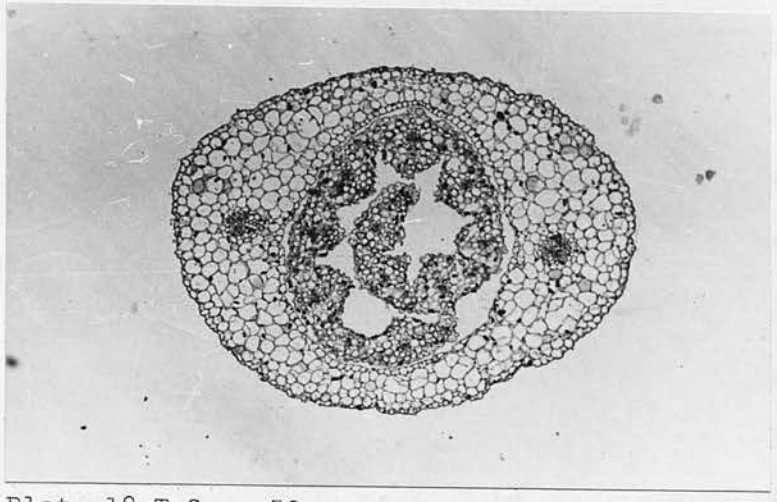


Plate 18 T.S. x 53

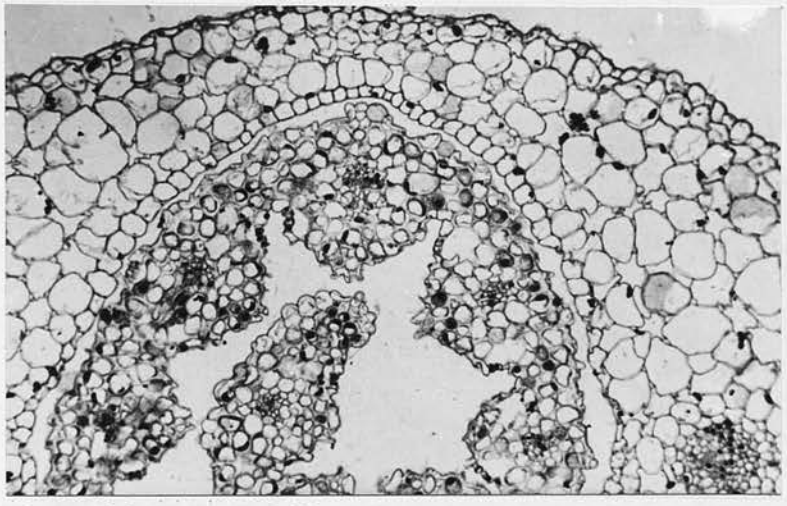


Plate 19 T.S. x 130

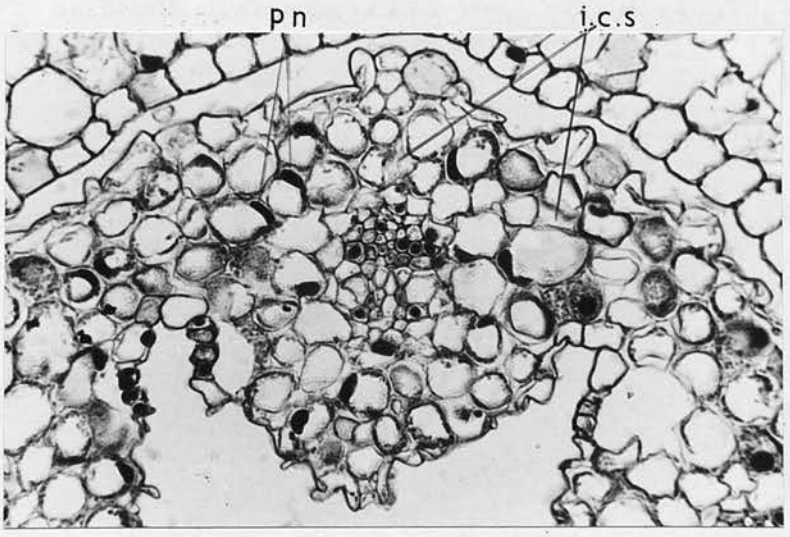


Plate 20 T.S. x 330

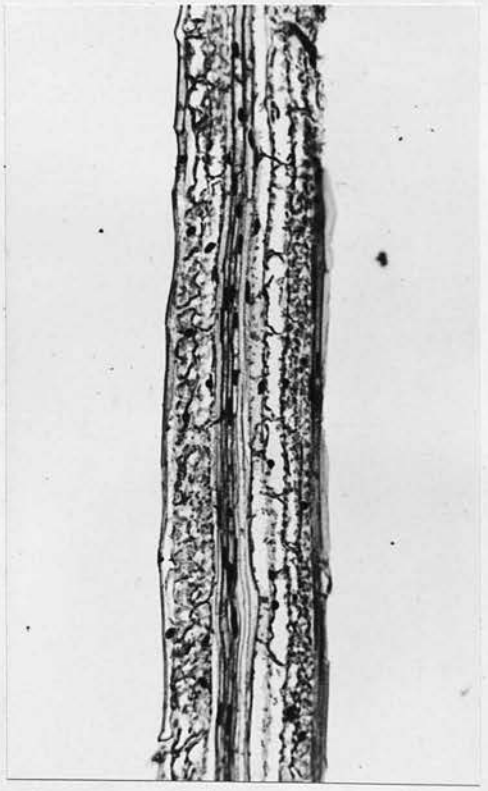


Plate 21 L.S. x 130

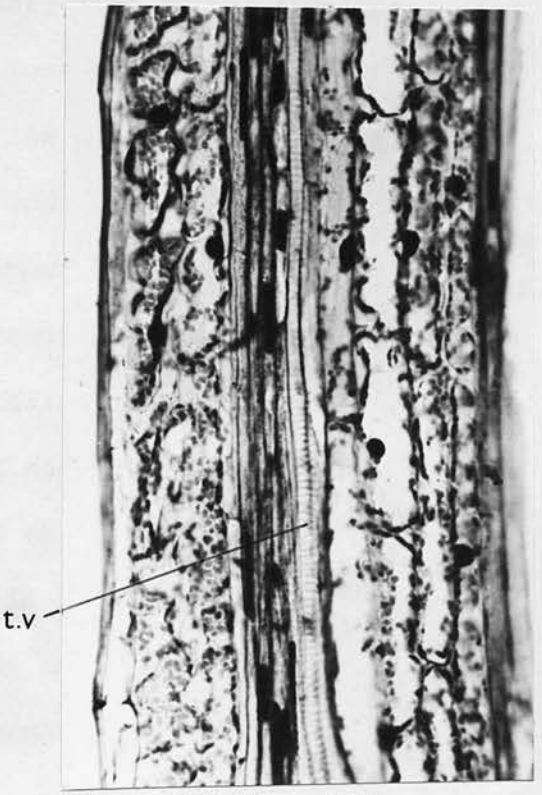


Plate 22 L.S. x 330

Plates 18-22 illustrate sections of S4 of Series 1 at 72 hrs.
p.n. peripheral nuclei, i.c.s. = intercellular space, t.v. thickened vessel.

In general the structure is similar to that of S3. In the epidermis, stomata and sub-stomatal cavities are present. All the bundles are more sharply differentiated than in S3, not only is the phloem well-developed but the xylem in this region contains fully mature tracheids and xylem vessels with spiral and annular thickening (Plate 22). A few cells with procambial appearance are present but these are less frequent than in S3. The mesophyll cells are large with peripheral nuclei and the sections suggest that these carry more chloroplasts than those of S3. The convolutions of the walls are possibly more pronounced and the intercellular spaces as seen in Plate 20 definitely larger.

Subsequent segments in the series have a structure virtually identical with S4 though the number of procambial elements in the xylem is reduced with further distance from the base of the leaf.

The series of observations indicate a progressive change from the base of the leaf to the apex though the major part of this change is complete by S4. In the base, the ridging of the mature leaf is present and each ridge is associated with a vascular bundle. The epidermis, although distinguishable from the mesophyll, consists of small cells which are non-vacuolate and contain large nuclei, stomatal initials are evident as slightly smaller and more deeply-staining cells. The mesophyll consists of vertical rows of small, non-vacuolate cells and the vascular bundles only contain procambial cells except in the case of the central bundle where at least one mature xylem vessel is present. With increasing distance from the base, the ridging becomes more prominent and the distinction between epidermis and mesophyll sharper with the epidermal cells expanding early.

The progressive differentiation of the stomatal complex is easily distinguished. In the bundles, the phloem and then the xylem differentiates, the vessels of the xylem being the last of all the tissues in the leaf to differentiate fully. In the mesophyll, the cells become larger and vacuolated with the walls highly convoluted, large intercellular spaces develop. With the progressive change in size, the number and prominence of the chloroplasts increases.

iii) Longevity of Meristem

The interpretation of data obtained from the 72 and 96 hr. leaves clearly depend on information being available on the relative activity of the basal meristem at the two times. It is important to have data on whether the meristem at 96 hrs. is ceasing to divide or whether it persists for some time beyond this point. Accordingly, observations were undertaken to determine the persistence of the meristem and the stage at which its component cells ceased to divide.

It was observed that the growth in length of the leaf ceased after 6 days, at this time the leaf had reached a length of about 13 cm. The cessation of growth could be due to either cell division ceasing in the meristem some days previously and the subsequent expansion of the cells of the meristem, or to the continued division of the meristem which later became dormant. It may be noted that the second condition has been observed in the intercalary meristems in the nodes of some monocotyledons (Begg and Wright 1962). However, it was considered more likely that the meristem would have completely expanded and the investigation was designed principally to investigate the timing of this.

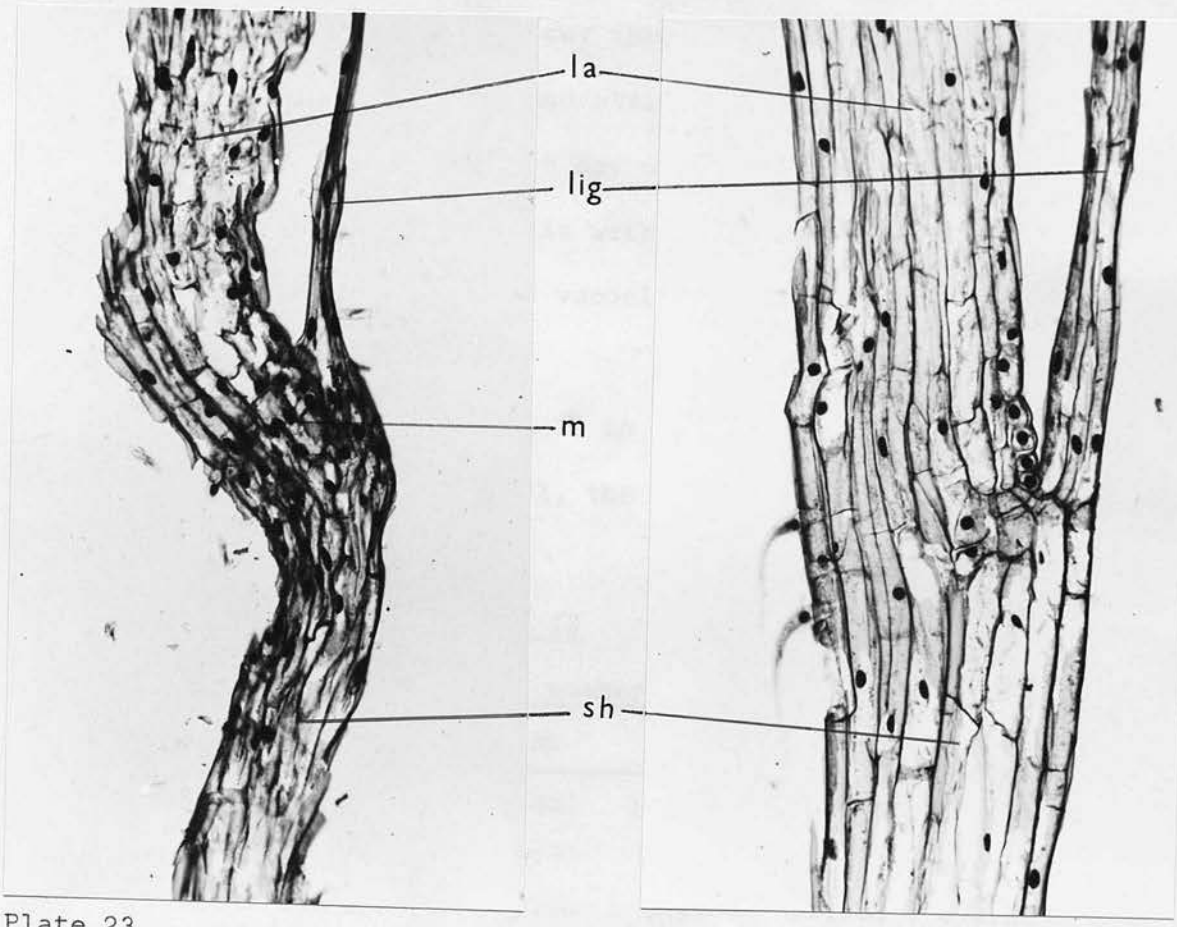


Plate 23

Plate 24

Plates 23 and 24 are L.S.'s through the region of the ligule of first leaves at 5 and 6 days x 220.
la = lamina, lig. = ligule, m = meristem, sh. = leaf sheath.

Two techniques were used, one was to cut sections from the appropriate regions of the leaf at different times and the other was to make cell counts at the base of the leaf as growth slowed down and ceased.

Longitudinal sections were cut through the region of the ligule of leaves aged from 5 to 10 days and stained with Delafield's Hematoxylin. It was observed that while the 5 day old leaf showed the presence of a few apparently meristematic cells with dense contents these had all disappeared by 6 days when only vacuolate, expanded cells were found. See Plates 23 and 24.

The results of counting cells in the basal 2 cm. of leaves aged from 3 to 7 days is shown in Table IX, the 2 cm. was divided into four segments of 5 mm.

TABLE IX

Cell numbers per segment

Segment No.	3 Day	4 Day	5 Day	6 Day	7 Day
Base 1)	223,000	234,400	143,500	29,600	28,500
2)	104,300	164,400	46,800	31,500	29,200
3)	44,300	46,100	34,600	31,800	29,100
4)	37,000	35,000	31,100	30,000	28,600

From the cell numbers of the basal segments shown in Table IX above it is evident that the cells of the meristem are fully expanded by Day 6. Further, the decrease in the cell number of the meristem is first shown from Day 4 to Day 5. It is, therefore, clear that at about 96 hrs., division in the meristem ceases and the expansion of the cells of the meristem begins.

The observations on the growth of the leaf at different ages confirm the conclusions of earlier workers that at a certain stage the cereal leaf grows by activity from a basal, intercalary meristem. At 24 hrs. the leaf is wholly meristematic though the cytological observations suggest that even at this stage mitotic activity is more intense at the base of the leaf than in the apical region. This tendency becomes more pronounced at 48 hrs. and by 72 hrs. cell production is restricted to the basal 5 mm. zone. This pattern persists until 96 hrs. after which the activity of the meristem declines. Thus, for the purposes of studying the progressive development of cells from the basal meristem, it is desirable to restrict attention to the stages subsequent to 48 hrs. In the 72 and 96 hr. leaves, the results indicate that much of the leaf has developed from the basal meristem and after 96 hrs. the further growth of the leaf is due principally to expansion of cells formed at an earlier stage. Thus, the general development of the leaf after the basal meristem has been differentiated necessarily involves two phases. A first phase from 48 to 96 hrs. and a second subsequent to 96 hrs. The incidence of these two phases has determined the development of this investigation into two groups of observations as described in the Introduction.

iv Cytological Observations

Since it had early been shown that the growth of the gramineous leaf was dependent, at certain stages, on the activity of an intercalary meristem at the base of the lamina (see Introduction), a survey of the cytological development of the first leaf at different stages was undertaken. However, in all the stages investigated, the meristem was active.

The results of these observations are reported below.

Observations were made on leaves 24, 48 and 72 hrs. after germination by the procedures described in Methods. The monocotyledonous leaf provides particularly good material for such observations, since cells formed in the intercalary meristem tend to remain in distinct rows for some distance. Thus by traversing single lines of cells, it is possible to determine the limits of division and extension. A particular study was made of the development of the stomatal complex since this had already been described for barley and some other monocotyledons by Stebbins and Shah (1960).

Observations on 24 hr. leaves

Preparations of these leaves showed that a very high proportion of the cells were meristematic (see Plate 25) and with the exception of the procambial elements all the cells were of approximately the same size. However, towards the apex there was some indication that cell expansion was beginning and although here, as in the rest of the leaf, cells were present in various stages of mitosis, mitotic figures were less frequent. At this stage in the growth of the leaf, there was no evidence to indicate the presence of separate marginal meristems as reported by Sharman and others (see Introduction) for some monocotyledons. It is, of course, possible that these had been active earlier but were no longer recognisable. The leaf was traversed from base to apex by procambial strands in which the cells were elongated in the axis of the leaf and had large nuclei. A central strand could be distinguished which carried, in addition to procambial elements, one or more fully differentiated vessels with spiral or annular thickenings. These vessels extended from the base of the leaf to the apex but the bars of thickening were closer together at the base.

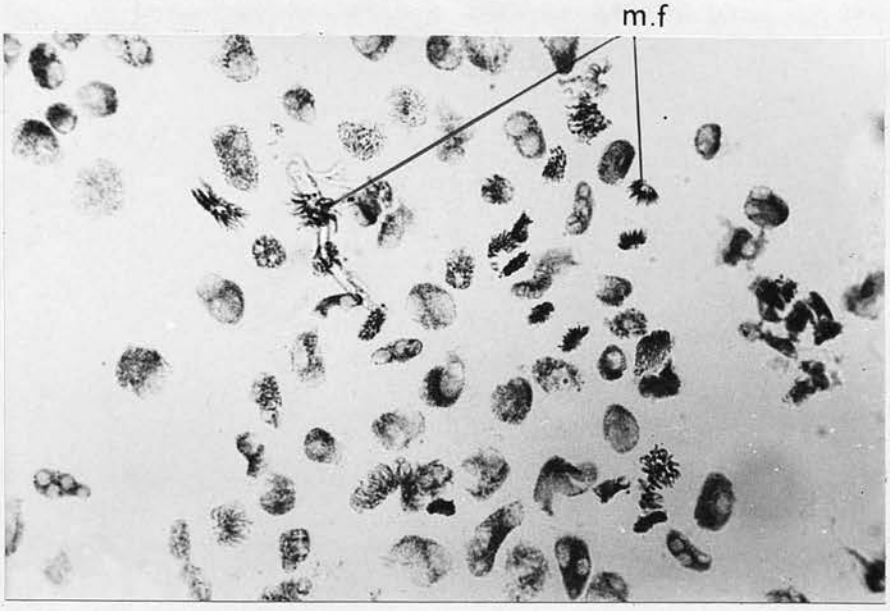


Plate 25

Squash preparation of meristematic region at the base of first leaf at 48 hours x 330.
m.f. = mitotic figures.

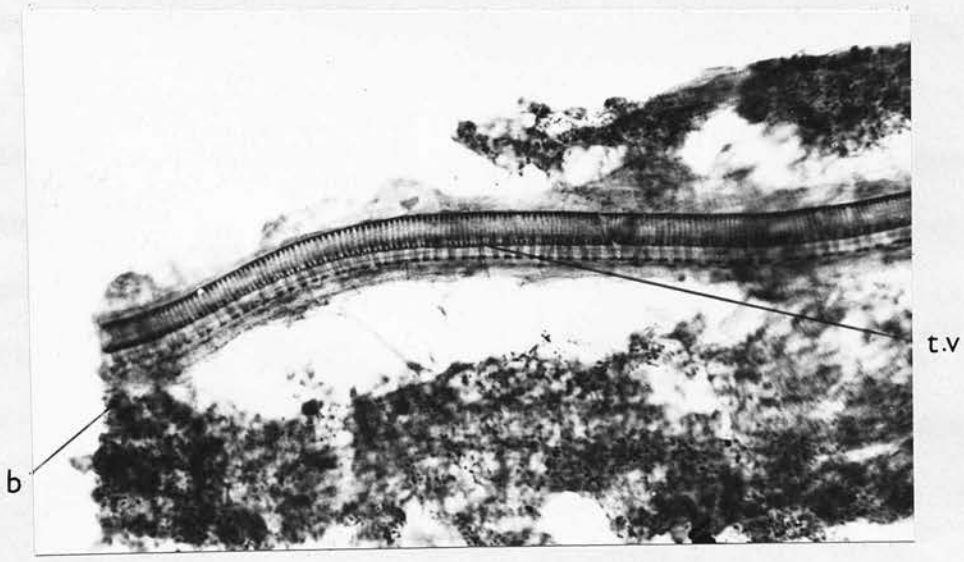


Plate 26

Squash preparation to show thickened xylem vessels at base of first leaf at 48 hours x 330.
b = base of leaf, t.v. thickened vessels.

This indicates that some cell extension was occurring towards the apex. Similar thickened vessels were also noted in some of the main lateral bundles. A preparation of such vessels at the base of the leaf is shown in Plate 26. Since this material was stained by the periodic acid-Schiff technique, the cell walls of the meristematic cells, which consist mainly of pectins and hemicellulose, cannot be distinguished. In soft squash preparations stomatal rows could be distinguished at the base of the leaf. These appeared as lines of small cells with densely staining nuclei. Towards the apex of the leaf, the rows of single cells were replaced by groups of four cells alternating with single cells. The detailed development of the stomatal complex is considered separately at the end of this section.

Observations on 48 hr. leaves

The 48 hr. leaf is approximately 1.2 cm. long and was dissected into two portions, a basal one of 5 mm. and an apical one of about 7 mm. In the extreme base of the leaf the appearance of the tissue was similar to that of the basal region of the 24 hr. leaf. In the apical zone of the basal 5 mm., however, the cells were larger and the early stages of expansion were apparent. In the basal portion, mitotic figures were frequent but in the apical portion none were seen. Here the cells were larger, more extensively vacuolated and had thicker cell walls. In this portion of the leaf a gradation in the staining properties of the nuclei from the base to the apex was noted. Towards the base, staining was dense while at the apex it was less pronounced.

At 48 hrs. the leaf is traversed by a number of procambial strands similar to those of the 24 hr. leaf, the central strand and some of the lateral ones were seen to contain fully differentiated xylem vessels. It was noted that while the bars of thickening were close together at the base, they were considerably further apart at the apex. The region in which the principal stretching of these vessels occurred was limited to between about 4.5 and 6 mm. from the base of the leaf. This suggests that the expansion process occurs fairly rapidly. Single rows of stomatal initials are present at the base of the leaf but by the beginning of the apical portion these consist of groups of four cells alternating with single cells, and at the apex of the leaf the two central cells have become fully differentiated guard cells.

Observations on 72 hr. leaves

The 72 hr. leaf was dissected into seven 5 mm. segments and each of these was investigated separately. In S1 the cytological features were similar to those observed in the 24 hr. leaf and the basal 5 mm. of the 48 hr. leaf. The general area of meristematic cells is larger at 72 hrs. and there is less evidence of elongation at the top of S1. Some mitotic figures are still present in S2 though cell expansion is evident in this segment. In S3 the cells are again larger and vacuolation is more advanced. No mitotic figures were observed and nuclear staining appears to be less intense than in S2. In S4 the cells are more or less fully expanded and the cytoplasm and cell walls are stained more deeply than in the basal segments. In S5, S6 and S7 the appearance of the cells changes little, though the cell walls of the mesophyll become more convoluted and nuclear staining continues to decrease.

The whole leaf is traversed by vascular elements, in the base these tend to be procambial strands with cells elongate in the long axis of the leaf and large densely-staining nuclei. At the level of S4 the procambial cells become replaced by fully differentiated tracheidal elements and xylem vessels and this tendency becomes more pronounced with increasing distance from the base. Even at the base of the leaf, some strands carry fully differentiated and thickened elements. The bars of thickening in these elements become widely separated as the cells extend in S2 and S3, it is significant that the elements differentiated from procambial cells at the level of S4 and S5 also have bars of thickening; however as extension has already occurred in these cells the bars remain close together. Rows of stomatal initials may be observed in the basal segment and fully differentiated stomata in S3. Transition stages are evident in the upper region of S1, in S2 and the lower part of S3.

The cytological observations recorded here amplify those made in the anatomical investigation. They confirm the general meristematic character of the young leaf and show that in the older leaf meristematic characteristics are confined to the base. It was recorded above that nuclear staining became less intense as distance from the base of leaf increased. In view of some results presented later on the DNA content of individual nuclei in Feulgen stained sections no explanation for this can be offered.

Development of the Stomatal Complex

The development of the stomatal complex in several monocotyledons, including barley, was investigated by Stebbins and Shah (1960). It was of interest to see to what extent the development differed in wheat, if at all. The observations described below were all made on 24 hr. leaves.

s.i. = stomatal initials

p. = procambial strand

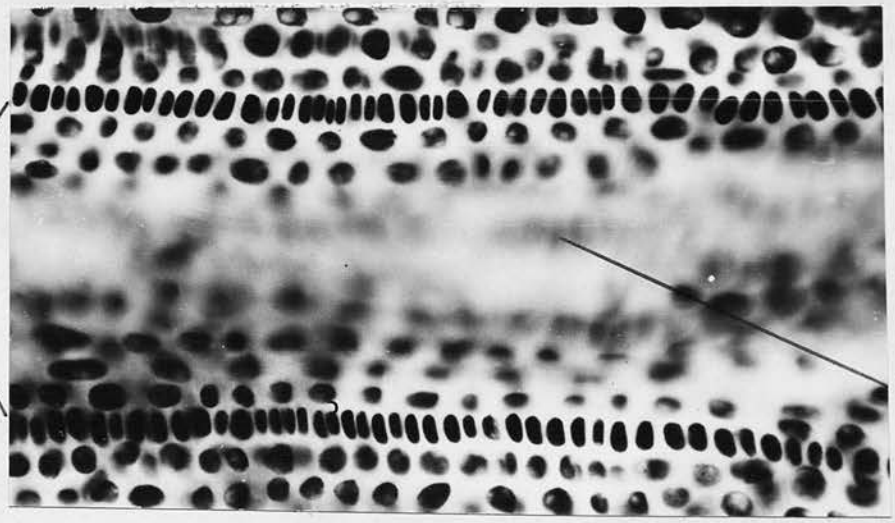
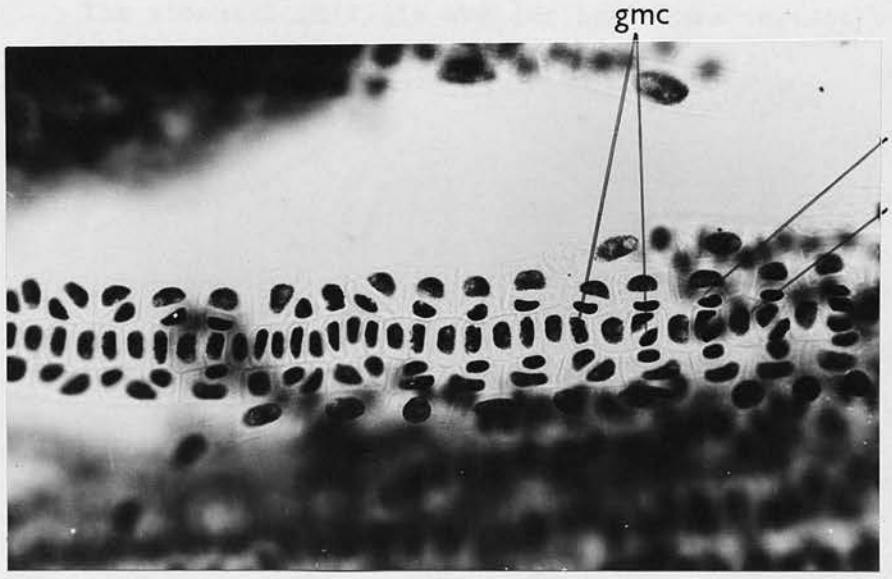


Plate 27 Single cell stage x 330



g.m.c. = guard mother cell

lr. = lateral row cell

e. = epidermal cell

Plate 28 3 to 4 cell stage x 330

st. = stomata

lr. = lateral row cell

e. = epidermal cell

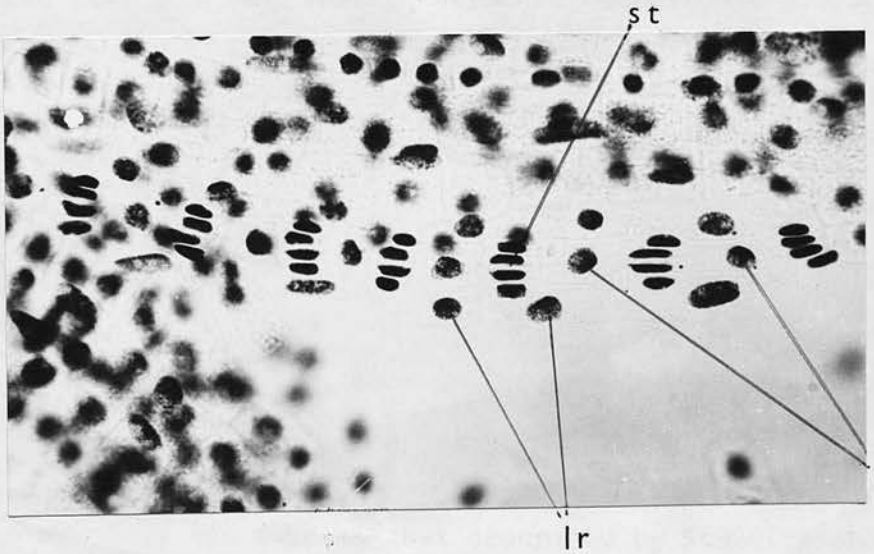


Plate 29 stomatal complex x 330

There are four stomatal rows per vascular strand, they are situated on both sides of the strand running parallel to it, two on the upper and two on the lower surface. The stomatal rows are laid down in the leaf primordia and are easily distinguished in Feulgen stained preparations of young leaves. At the base of the leaf, the stomatal initials are seen as a single row of small cells with densely-staining nuclei. These nuclei frequently appeared to be elongate at right angles to the axis of the leaf. Plate 27 shows two rows of stomatal initials at the base of the leaf, between them, out of focus, is a procambial strand. The stomatal initials are further characterised by the presence on either side of them of two closely associated rows of epidermal cells, the lateral rows. These cells tend to remain rather smaller than typical epidermal cells.

The stomatal initials divide once in the axis of the leaf giving rise distally to small cells with heavily-staining nuclei and proximally to slightly larger cells with less heavily-staining nuclei. The distal cells are the guard mother cells (GMC's) and the proximal ones develop into the normal epidermal cells which separate the stomata in the mature leaf. The next divisions occur in the lateral rows, these contain half as many cells as the stomatal rows and they undergo asymmetrical mitoses producing a subsidiary cell on each side of each GMC. These divisions are illustrated in Plate 28. They give rise to the three-celled stage of Stebbins and Shah. The final division is that of the GMC's at right angles to the axis of the leaf, also shown in Plate 28, giving the four-celled stage. The nuclei of the stomatal complex undergo considerable elongation during the subsequent growth of the leaf (Plate 29) and the cells differentiate to form the typical stomata of the cereals. Thus it is evident that the development of the stomatal complex in wheat is the same as that described by Stebbins and Shah for barley.

b) Quantitative Characteristics

The quantitative characteristics of the system are presented in two groups corresponding to the two phases of the investigation. The characteristics of the 72 and 96 hr. leaves are considered first and, subsequently, the changes in selected regions of the leaf from 3 to 10 days. With both groups the original data is expressed in tabular form in the Appendix while graphs of mean data are given in the text.

Series 1

The main body of observations involve determinations of cell number, fresh weight, dry weight, total nitrogen, TCA soluble and TCA insoluble nitrogen, and chlorophyll content. The primary values are given in Fig.10 while the corresponding derived values on a unit cell basis are given in Fig.11. The scale in the figures is the same for the 72 and 96 hr. leaves but since the 96 hr. leaf has 14 segments and the 72 hr. leaf only 7, the termination of the curves with the two leaves is necessarily different. The curves for the 72 hr. leaves have been drawn on transparent paper and mounted above the 96 hr. curves using common axes. A blank sheet has been inserted between the two figures so they may be examined separately or in conjunction with one another.

Cell Number

The number of cells per segment (Fig.10) falls for the 72 hr. leaf from 210,000 to 40,000 over the first three segments. From S4 to S6 the number is more or less constant around 30,000 with a slight decrease in S7 due to tapering.

The high basal values undoubtedly reflect the fact that these segments are composed, principally, of meristematic cells while the smaller numbers in the apical half of the leaf show that this is composed mainly of large, vacuolated cells. The curve for 96 hrs. again shows a decrease from the first to the fourth segment with a more or less constant number from this to the apex. There is a significant increase in the number of cells in S2 from 72 to 96 hrs. This is caused by an increase in meristematic activity resulting in the zone of cell expansion spreading further in the apical direction.

Fresh Weight

The curves for fresh weight per segment (Fig.10) show that at 72 hrs. there is an increase from 1.45 to 1.86 mg. over the first three segments and thereafter weight remains more or less constant until the tapering of the leaf at the tip. At 96 hrs. the change in fresh weight along the course of the leaf is very similar there is an increase from 1.26 to 1.80 mg. from S1 to S6 and thereafter values are more or less constant until tapering begins at S13. The fresh weight values for the lower part of the 96 hr. leaf are smaller than those for the same zone of the 72 hr. leaf, the difference is of the order of 16% and is probably due to the necessary difference in the preparation time for the segments from the two leaves. The 72 hr. leaf only involves 7 segments and dissection, therefore, occupies a shorter time than that required by the 14 segments of the 96 hr. leaf. Thus, with the 96 hr. leaf, a longer time during which evaporation can occur is involved and this may have been the origin of the fresh weight difference. The data for fresh weight per cell (Fig.11) show that the value at 72 hrs. rises from 0.7 mg. x 10^{-5} to 5.5 mg. x 10^{-5} over the first four segments and from S4 to S7 remains more or less constant. The low values in S1 and S2 are undoubtedly due to the fact that these are composed wholly or partly of small meristematic cells.

The increase to the fourth segment accompanies the increase in volume and consequent water accumulation with continuous expansion. At 96 hrs. the values per cell increase sharply from S1 to S5 and then less steeply from S5 to S13. The increase from the fifth to the thirteenth segment is from 5.3 mg. x 10^{-5} to 7.1 mg. x 10^{-5} this being an increase of about 24%. Again the low values of the basal segments indicate the meristematic nature of the cells, while the steady increase thereafter reflects progressive cell expansion. It is clear, however, that this expansion probably occurs in two phases. After division has occurred in the meristem, vacuolation begins and since the number of cells per segment is decreasing from the first to the fifth segment, this necessarily implies that the expansion consequent on vacuolation involves an increase in the length of the cells. At the same time, it is significant that from the first to the fifth segment the fresh weight per segment is also increasing. This suggests that while the cells are increasing in length they are also increasing in width. This conclusion is supported by direct cytological and anatomical observations. From the fifth to the thirteenth segment, there is only a slight decrease in the number of cells per segment, suggesting that after S5 there is no further increase in the length of individual cells. On the other hand, from S5 to S13 there is a considerable increase in fresh weight per cell. Thus, over the interval represented by this gap, cell expansion continues in a lateral direction. The first phase of expansion involves increases both in length and width of the cells while the second phase involves increases in width only. The apparent slight decrease in the number of cells between S5 and S13 is probably an artefact. As they become older cells become more friable and more susceptible to disruption in the maceration procedure.

A change in width, after length increase has ceased, is not shown decisively by the data for the 72 hr. leaf. But, in this leaf the number of values available after the fourth segment is limited and there is some evidence of an increase in fresh weight per cell from S4 to S5. Thus, the data for the 72 hr. leaf, while they do not confirm the conclusion drawn from the 96 hr. data, are nevertheless compatible with them.

Dry Weight

The values for dry weight per segment (Fig.10) show that both at 72 and 96 hrs., there is little change in dry weight from the base to the apex of the leaf. The values for dry weight per cell (Fig.11) show that at 72 hrs. dry weight increases progressively from S1 to S7. Similarly, the curve for 96 hrs. shows a corresponding progressive increase from S1 to S14. In both leaves the increase is most pronounced from S2 to S4. It is evident that dry weight increases as expansion proceeds, the greatest increase occurs during the rapid expansion in length but a slower increase in dry weight is maintained during the phase of expansion in width only. There is some indication that dry weight increase may continue even after the width increase has ceased.

Total Nitrogen

The values for total nitrogen per segment (Fig.10) show that with the 72 hr. leaf the value is highest in the basal segment; it decreases from this to S3 but thereafter increases slightly to S6. With the 96 hr. leaf the value is again highest in S1, decreases from this to S5 and then rises slowly to S12 dropping again at the apex. Total nitrogen per cell (Fig.11) at 72 hrs. is lowest in the basal segment and rises steadily to S7, the rise being sharpest from S1 to S4. At 96 hrs., the lowest value is again in the basal segment and there is a steady rise to S13, the rise being particularly sharp from S1 to S3.

Quantitative Characteristics per Segment: 72 hrs

Key as for 96 hrs

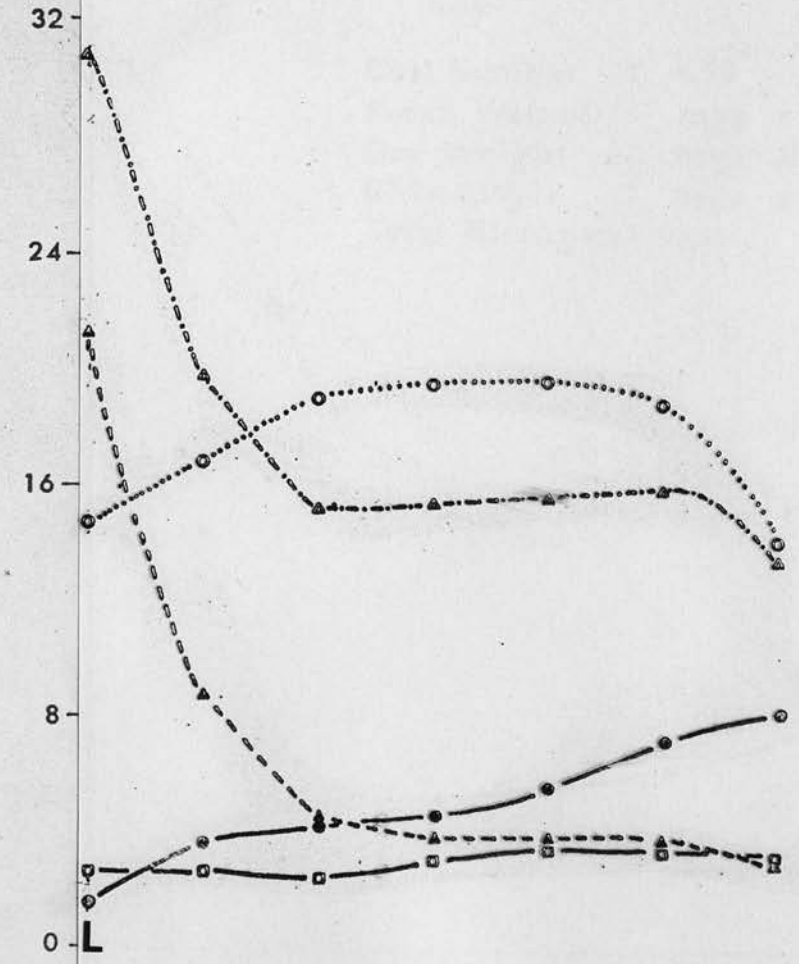


Fig. 10

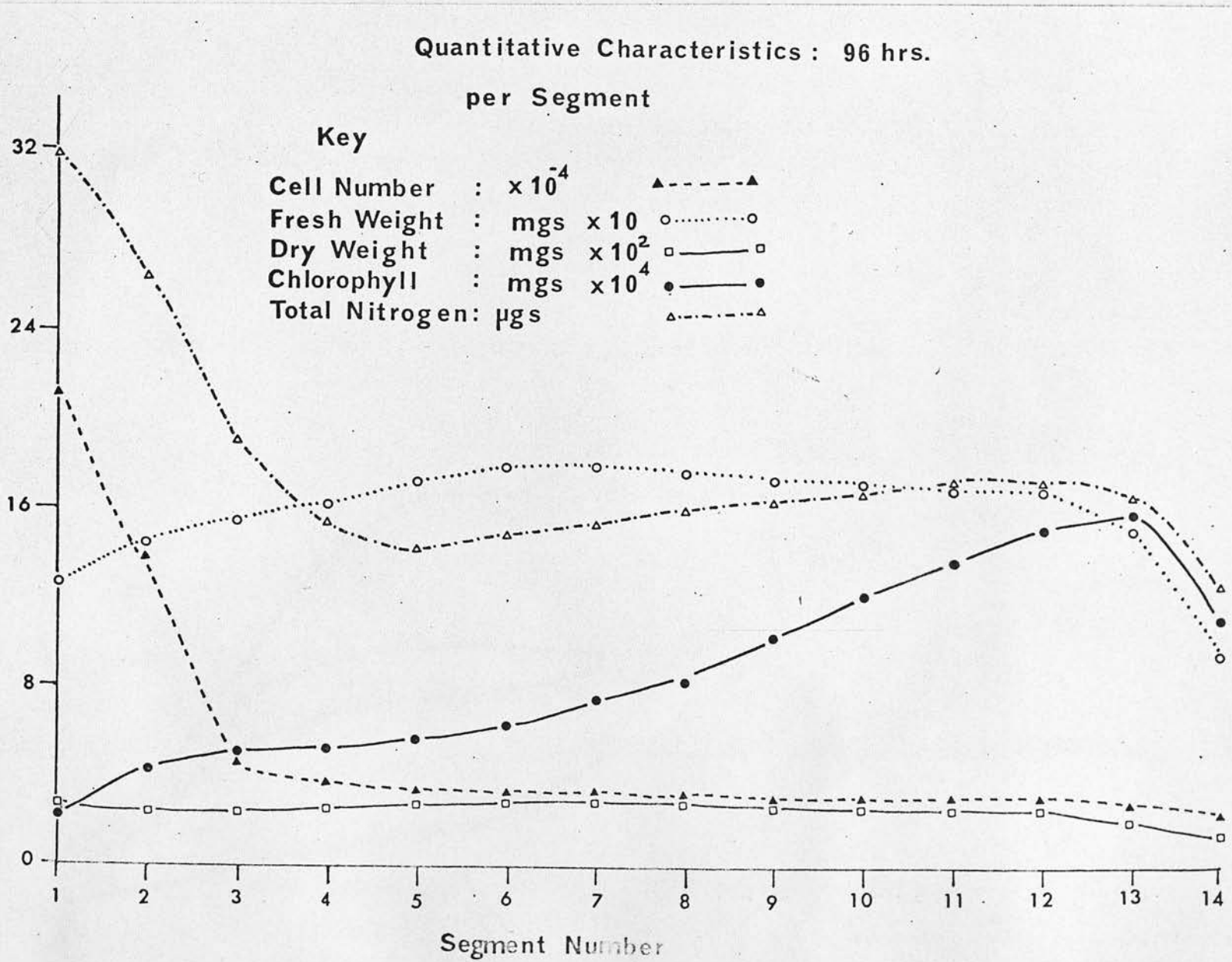

Quantitative Characteristics : 96 hrs.
per Segment
Key
Cell Number : x 10^-4
Fresh Weight : mgs x 10
Dry Weight : mgs x 10^2
Chlorophyll : mgs x 10^4
Total Nitrogen: µgs
32
24
16
8
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number

Fig.10

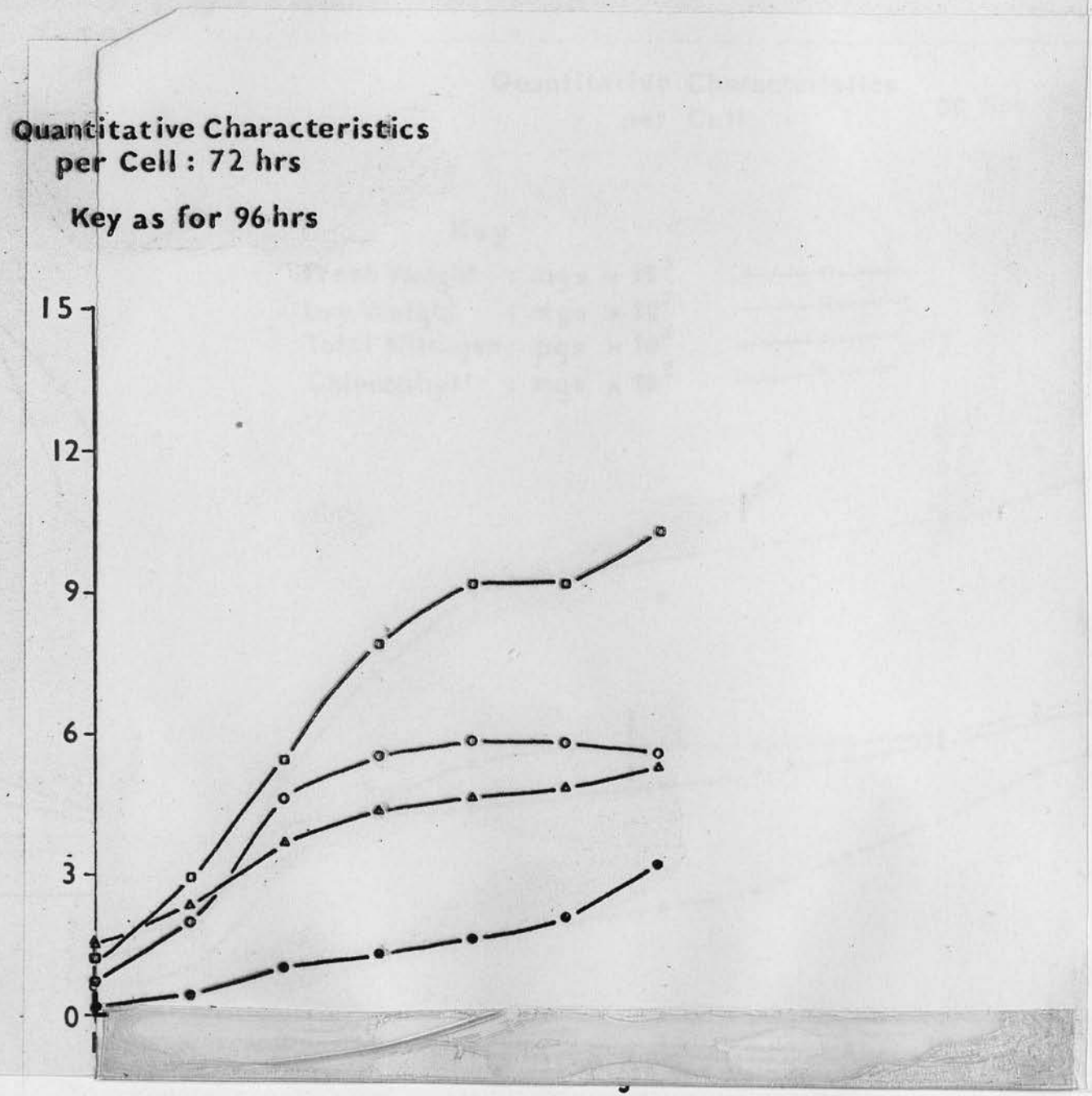

Quantitative Characteristics
per Cell : 72 hrs
Key as for 96 hrs
15
12
9
6
3
0

Fig.11

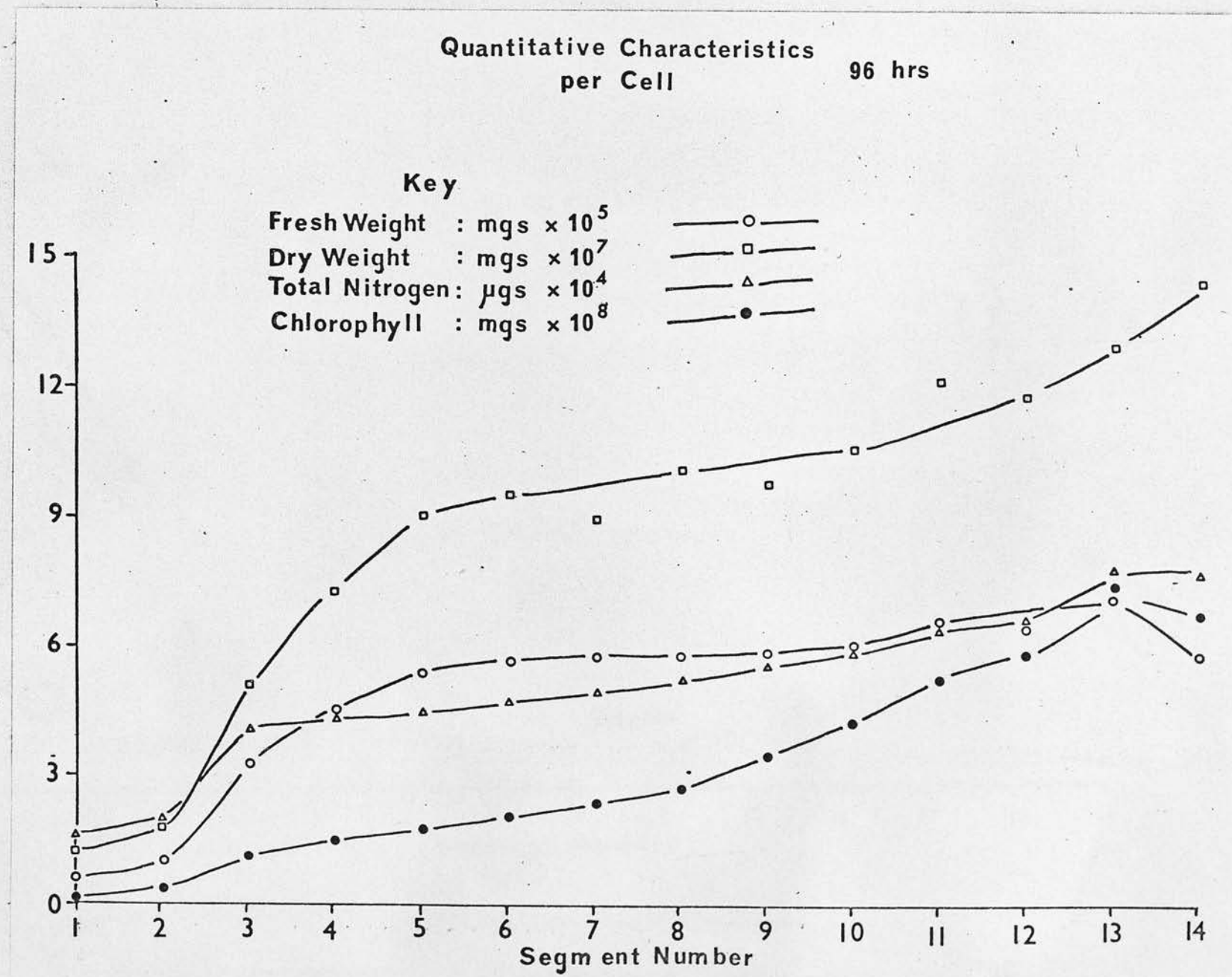

Quantitative Characteristics
per Cell
96 hrs
Key
Fresh Weight : mgs x 10^5
Dry Weight : mgs x 10^7
Total Nitrogen : µgs x 10^4
Chlorophyll : mgs x 10^8
15
12
9
6
3
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number

Fig. 11

T.C.A. Soluble and Insoluble Nitrogen Series 1

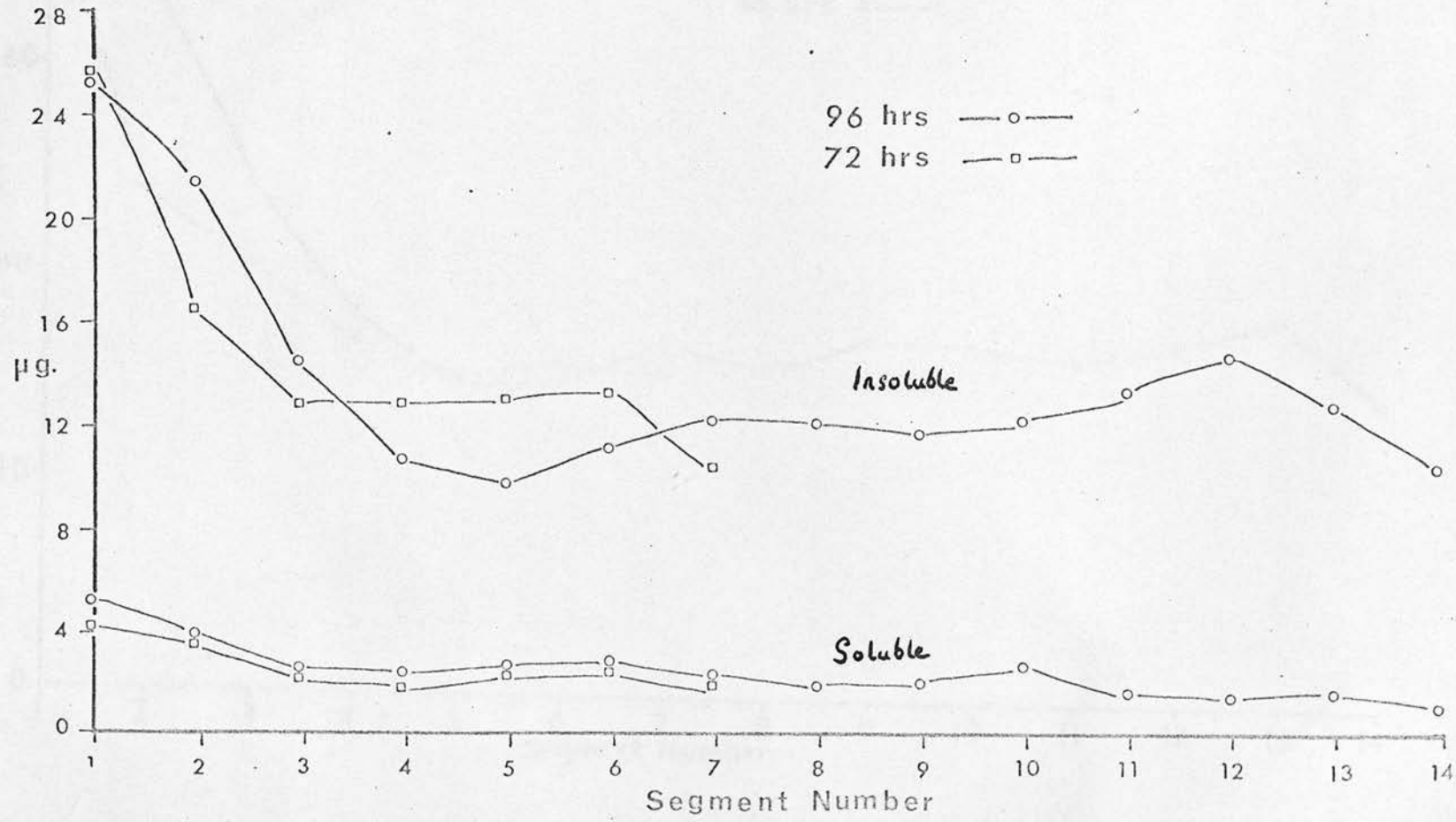


Fig.12

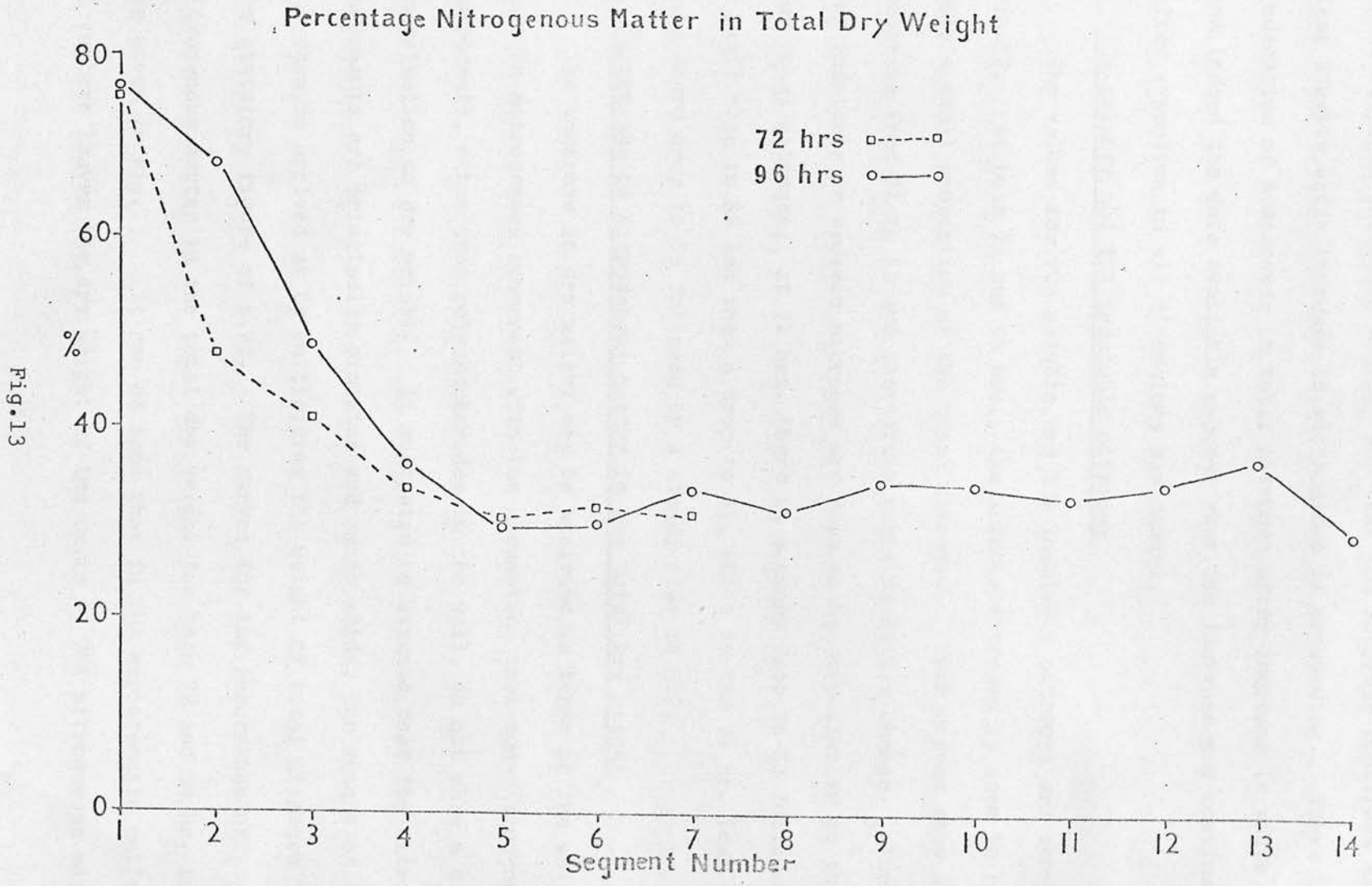

Percentage Nitrogenous Matter in Total Dry Weight
72 hrs
96 hrs
80
60
40
20
0
%
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number

Fig.13

The data indicate that while expansion is occurring in all dimensions, total nitrogen increases sharply and that the increase continues although less steeply while increase in width alone is proceeding. There is no indication of a decrease in total nitrogen after increase in width ceases and indeed the data available suggest that the increase may continue after expansion in all dimensions has ceased.

TCA soluble and TCA insoluble nitrogen

The values for TCA soluble and TCA insoluble nitrogen are shown in Fig.12. At both 72 and 96 hrs., the soluble nitrogen is seen to represent only a small proportion of the total nitrogen. Both curves show a slight decrease from S1 to S3 and thereafter there is little change. The curves for insoluble or protein nitrogen are seen to be very similar to those for total nitrogen; at 72 hrs. there is a sharp drop to S3 followed by a small rise to S6 and then a drop to S7, while in the 96 hr. leaf there is a sharp drop to S5 followed by a steady rise to S12.

Percentage of Nitrogenous matter in the Total Dry Weight

The increase in dry weight may be analysed in terms of the wall component and the nitrogenous component with the assumption that non-nitrogenous components, other than polysaccharides in the wall, do not make a significant contribution to dry weight. It must also be assumed that the nitrogenous components are principally proteins and amino acids, the weight of these may then be arrived at by multiplying the weight of total nitrogen by the statutory figure of 6.25. The curves for the percentage of nitrogenous matter in the total dry weight for both 72 and 96 hr. leaves are given in Fig.13. It can be seen that in the meristematic cells of S1 in both leaves the dry weight of the cells is 76% nitrogenous matter.

As the total dry weight increases, so the percentage due to nitrogenous matter decreases; initially this occurs rather more slowly at 96 hrs. than at 72 hrs. However, at S5 in both leaves the lowest value 30% is reached. After this, at 96 hrs., there is a slow rise to 36% at S13. In the initial sharp increase in dry weight per cell from S1 to S4 approximately 70% of the increase is due to increase in wall weight and only 30% to increase in nitrogenous matter. In the subsequent gradual increase, 48% of the increase is due to nitrogenous matter.

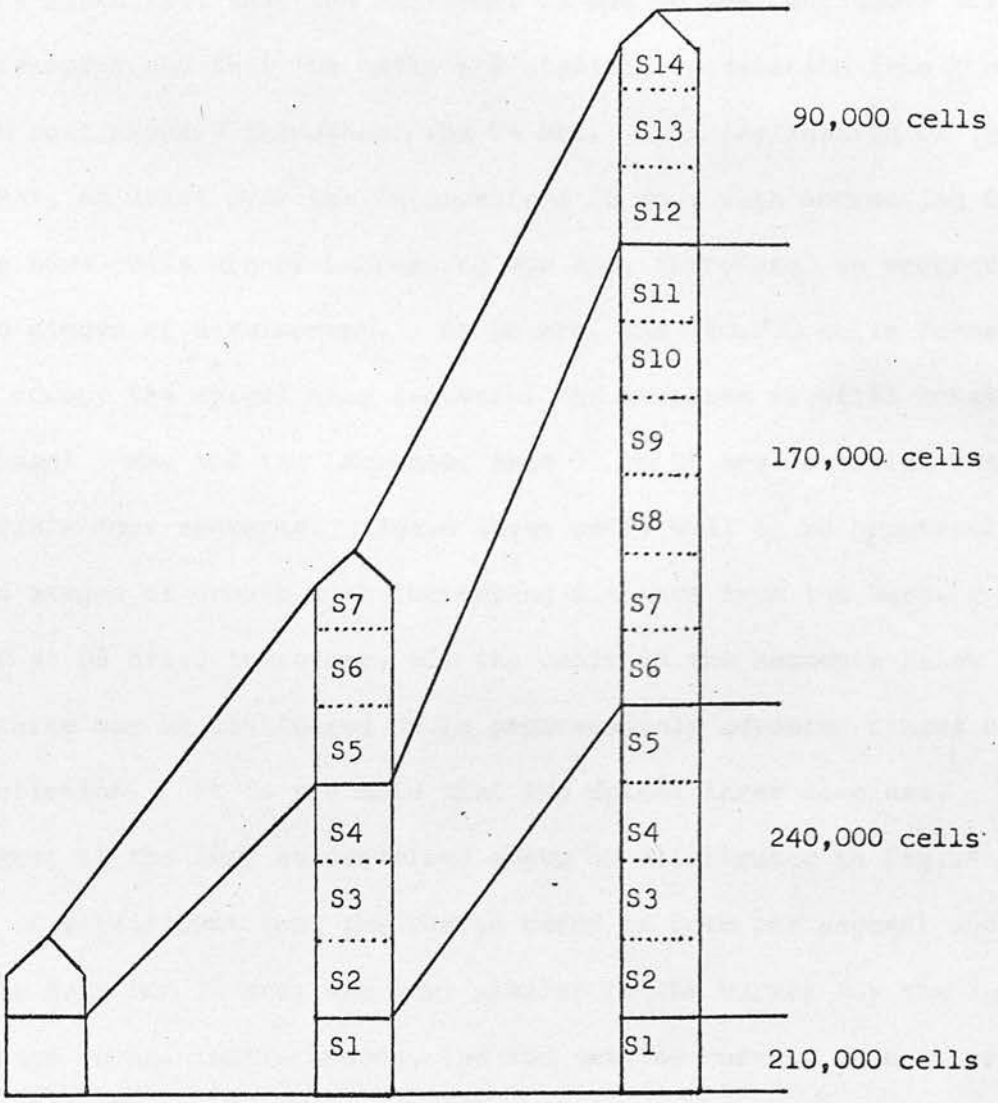
Chlorophyll content

The values for total chlorophyll per segment (Fig.10) and total chlorophyll per cell (Fig.11) show, in all cases, a steady rise from the base of the leaf, which is almost colourless, to the apex. The figures for chlorophyll (a) and chlorophyll (b) were calculated separately and are shown in the Appendix together with the ratio of Ca to Cb. This ratio was found to be approximately 2:1 for the basal two segments in both leaves and more or less constant at 3.5:1 for the rest of the leaf. It is significant that the proportion of chlorophyll weight to the weight of total nitrogenous matter increases throughout the length of the leaf. The increase is about tenfold in the 72 hr. leaf and about fifteenfold in the 96 hr. leaf. This probably indicates that there are few, if any, chloroplasts in the meristematic cells at the base of the leaf but that the number and size of chloroplasts increases steadily with age. This is also suggested by the anatomical data. It is of interest that, particularly in the 96 hr. leaf, the increase in chlorophyll content is gradual and progressive and there is no abrupt change in the position at any point. At 96 hrs., the coleoptile envelopes the lower 3 cm. or six segments of the leaf and an abrupt change in the rate of accumulation of chlorophyll might be expected at the point where the tissue ceases to be covered.

It is clear, however, that this does not occur and it may be concluded that the presence of the coleoptile does not significantly affect the development of the photosynthetic mechanism.

Discussion

The data of this section are of some significance in relation to the development of the leaf as a whole. It may be recalled that at 24 hrs. the whole leaf, with the exception of some xylem elements, is composed of non-vacuolate meristematic cells. Even at this stage, however, when the leaf is only 5 mm. long, there are some indications of vacuolation beginning at the apex. By 48 hrs. a basal intercalary meristem has been developed; this is no doubt a consequence of progressive vacuolation from the apex which ceases 4 mm. from the base of the leaf and leaves an active meristem in this region. At 48 hrs. there are approximately 160,000 cells in the meristem (basal 5 mm.) and 90,000 cells above it in the terminal 7 mm. At 72 hrs. the total number of cells has increased to 470,000, therefore, in the intervening 24 hrs. there has been an increment of 220,000 cells. Since the meristematic basal 5 mm. at 72 hrs. contains 210,000 cells the increment in vacuolated cells in the previous 24 hrs. has been 170,000 cells. This increment has occurred between the original 90,000 vacuolate cells and the meristem at the base. Since it has been established that in fully-expanded tissue there are approximately 30,000 cells per 5 mm., the 90,000 cells present in the apical 7 mm. at 48 hrs. will occupy the apical 15 mm. of the leaf when fully extended at 72 hrs. The meristem occupies the basal 5 mm., thus the increment of 170,000 cells occupies the intermediate 15 mm.



48 hrs.	72 hrs.	96 hrs.
Length 10 mm.	Length 35 mm.	Length 70 mm.
250,000 cells	470,000 cells	710,000 cells

Fig.14 Diagram showing the derivation of the segments of the leaf used in the experiments of Series 1.

It may be emphasised that the increment is due to the continuous activity of the meristem and that the cells are starting vacuolation from the meristem continuously throughout the 24 hrs. The implication of this being that, at least over the intermediate 15 mm., with increasing distance from the base cells are of increasing age and, therefore, in progressively advanced stages of development. At 96 hrs. the 260,000 cells formed by 72 hrs. occupy the apical nine segments, the meristem is still considered as the basal 5 mm. and the increment from 72 to 96 hrs. occupies the intermediate four segments. Again these cells will be in progressively advanced stages of growth with increasing distance from the base. In the leaf at 96 hrs., therefore, all the cells in the segments below the apical three may be considered as in progressively advanced stages of differentiation. It is probable that the apical three also are. The development of the leaf as described above is illustrated in Fig.14.

It is significant that the curves based on both per segment and per unit cell data for 72 hrs. are very similar to the curves for the lower part of the 96 hr. leaf; indeed, the two sets of curves, when superimposed, show a very close coincidence. This suggests that the development of cells between 48 and 72 hrs. and 72 and 96 hrs. has, at least in quantitative characteristics, been very similar.

Series 2

The same characteristics were investigated for Series 2 as for Series 1 with the addition of determinations of nucleic acid content. As described under 'Material and Methods', leaves aged from 3 to 10 days were used, four 5 mm. segments being cut on each day.

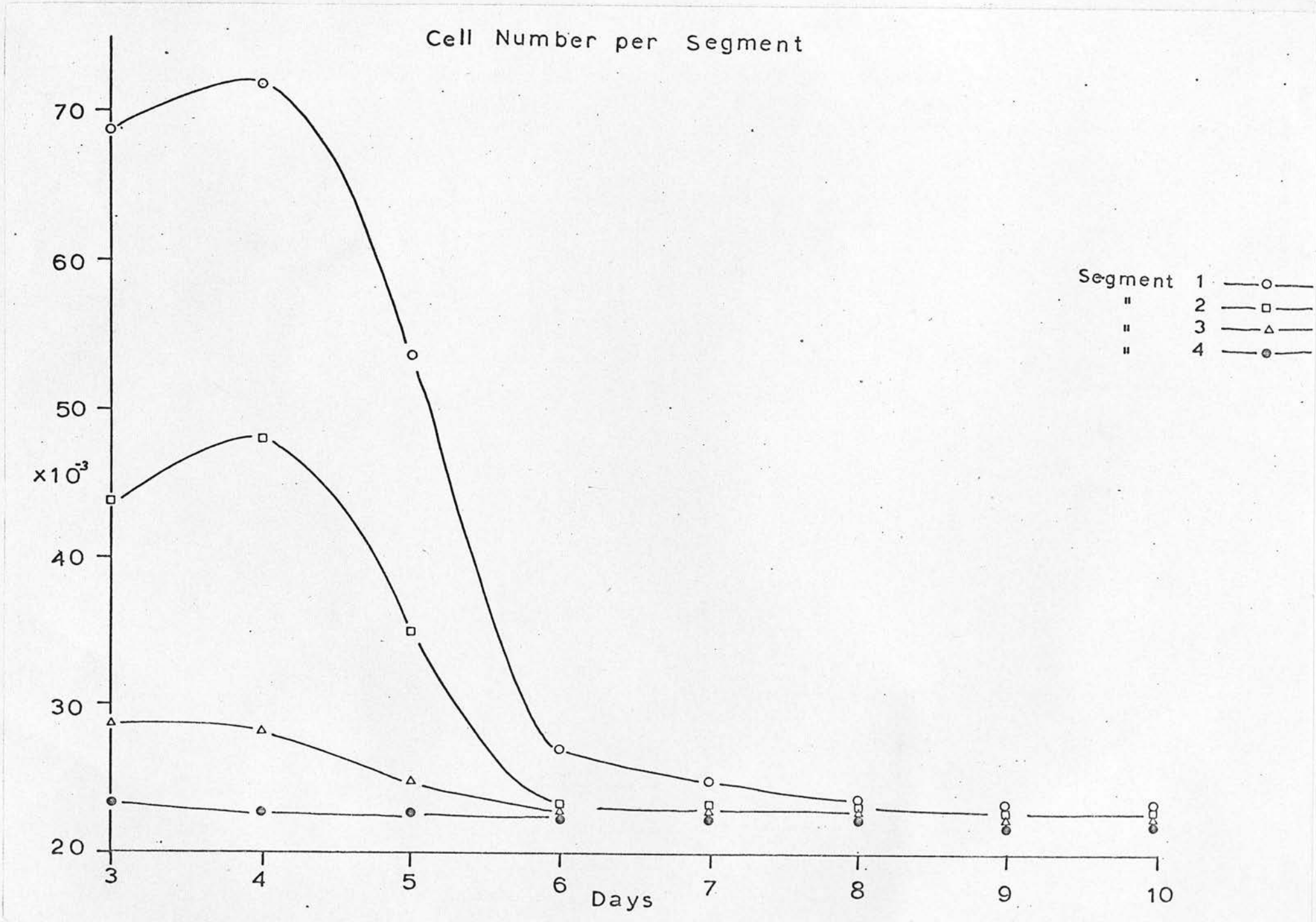

Cell Number per Segment
70
60
50
40
30
20
x10⁻³
3
4
5
6
7
8
9
10
Days
Segment 1
" 2
" 3
" 4

Fig.15

Three segments were taken serially from the base of the leaf and these are referred to as S1, S2 and S3, and one from a subapical position referred to as S4.

The results are again presented graphically in the text and as tables in the Appendix. In this series, however, the curves for individual segments are plotted against time in days.

Cell number

The data showing the numbers of cells in the selected segments on the different days are shown in Fig.15. In S1 cell number shows a slight rise from the third to the fourth day and this rise is probably significant, it indicates that during this interval there has been rapid cell division with consequent reduction of the average volume of cells in the segment. After the fourth day the number of cells drops sharply to the sixth day and thereafter it decreases more slowly but still significantly to about day 8. Evidently, cell division declines sharply after the fourth day and the cells immediately start to expand and continue to expand over the next 48 hrs. Further expansion occurs but at a much slower rate after the sixth day. In the second segment there is a slight rise in the number of cells from the third to the fourth day and thereafter there is a sharp decrease to the sixth day with no further change after this time. On the third day the cells of the second segment are larger than those of the first and there are fewer cells in the segment than in the first. The rise between the third and fourth days is no doubt due to rapid meristematic activity principally in S1. This causes a large number of small cells to be cut off in to S2 and thus reduces the average cell volume and increases the cell number in the segment.

Cell division declines sharply at this stage and over the next 48 hrs. rapid expansion occurs with a consequent decrease in the number of cells per segment. In S3 the number of cells present on the third day is less than in S2, this being a reflection of the fact that the cells in this segment are larger. From the third to the sixth day there is a progressive decrease in cell number, indicating a progressively larger volume in the component cells. In S4 there is no change in cell number throughout the experimental period. This means that these cells must have completed their extension in length by Day 3.

The change in the number of cells in S1, S2 and S3 requires some comment. It is significant that in all three segments cell number continues to decrease over the same interval of day 4 to day 6. Cell division in the basal segment having declined sharply after the fourth day. It may be emphasised that the tissue being sampled on successive days is not, of course, the same; many cells in S2 for instance will have been derived from S1 as a result of expansion in the previous 24 hrs. In view of this, it is clear that the continuous decrease in the number of cells from the fourth to the sixth day does not necessarily indicate that the extension of a single cell occupies more than 24 hrs. It is clear that once division has begun to decline in the meristem, vacuolation does not occur simultaneously in all the component cells. The data indicate that in the basal segment, vacuolation begins in the distal end and spreads progressively downwards towards the base. The consequence of this must be that cells are displaced progressively into S2 and S3 and the process of displacement together with that of extension occupies fully 48 hrs.

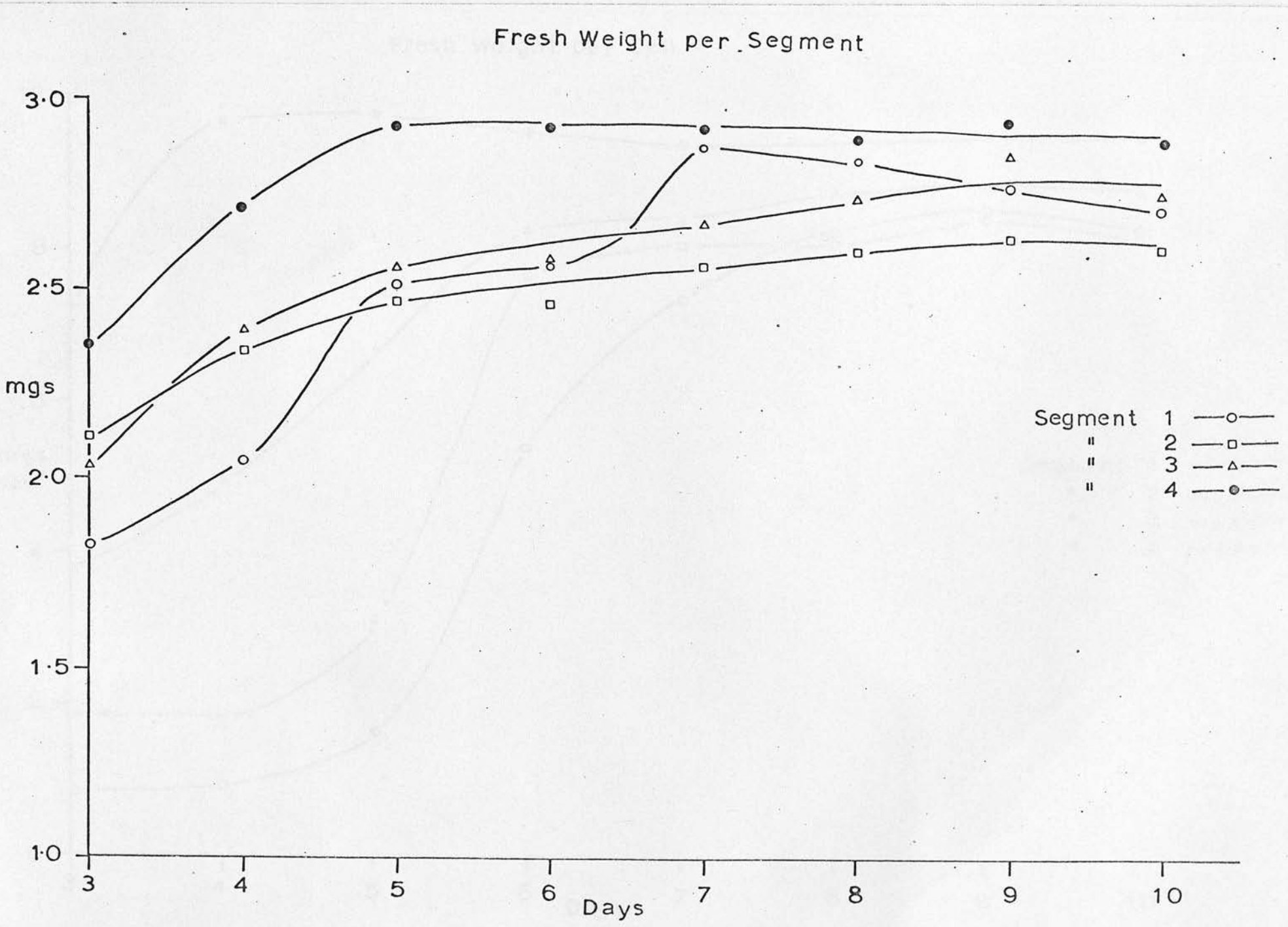

Fresh Weight per Segment
3·0
2·5
2·0
1·5
1·0
mgs
3
4
5
6
7
8
9
10
Days
Segment 1
" 2
" 3
" 4

Fig.16

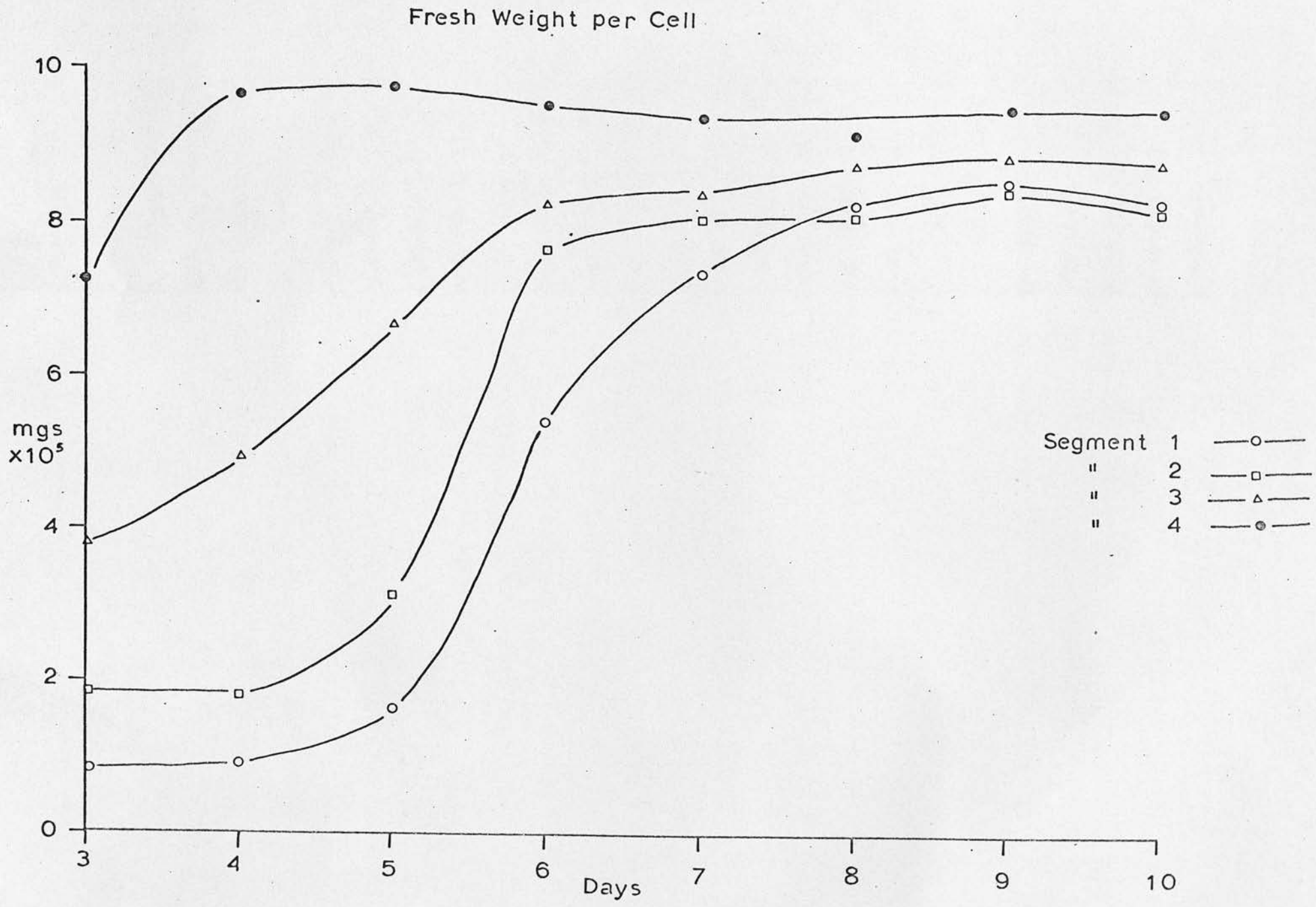

Fresh Weight per Cell
10
8
6
4
2
0
mgs
x10⁵
3
4
5
6
7
8
9
10
Days
Segment 1
" 2
" 3
" 4

Fig.17

A further consequence of this series of events may be noticed, it is that the time course in change of number of cells in the basal three segments will inevitably be dominated by the successive vacuolation of the cells of S1. Thus in the first three segments the cell number will reach a constant value at approximately the same time; the data show that this time is six days.

Fresh Weight

The change in fresh weight with time for each of the segments of the experimental series is shown in Fig. 16. With the first three segments fresh weight increases markedly during the first six to seven days and thereafter remains more or less constant. With S4 fresh weight increases from the third to the fifth day and then remains constant.

It is significant, in this connection, that in S1, S2 and S3 the number of cells in the segments decreases between the fourth and sixth days. In view of this, the increase in fresh weight in these three segments is no doubt due to an increase in thickness or width or both, of the leaf. This, in turn, implies an increase in the dimensions of the individual cells. In S4 there is no change in cell number with time, nevertheless there is a significant increase in fresh weight between day 3 and day 5; again this implies an increase in leaf width consequent on a change in the width of the cells.

The changes in fresh weight per cell are shown in Fig.17. In S1 and S2, there is no change between the third and fourth days; this corresponds with the slight increase in the number of cells in these segments shown by the data in Fig.15.

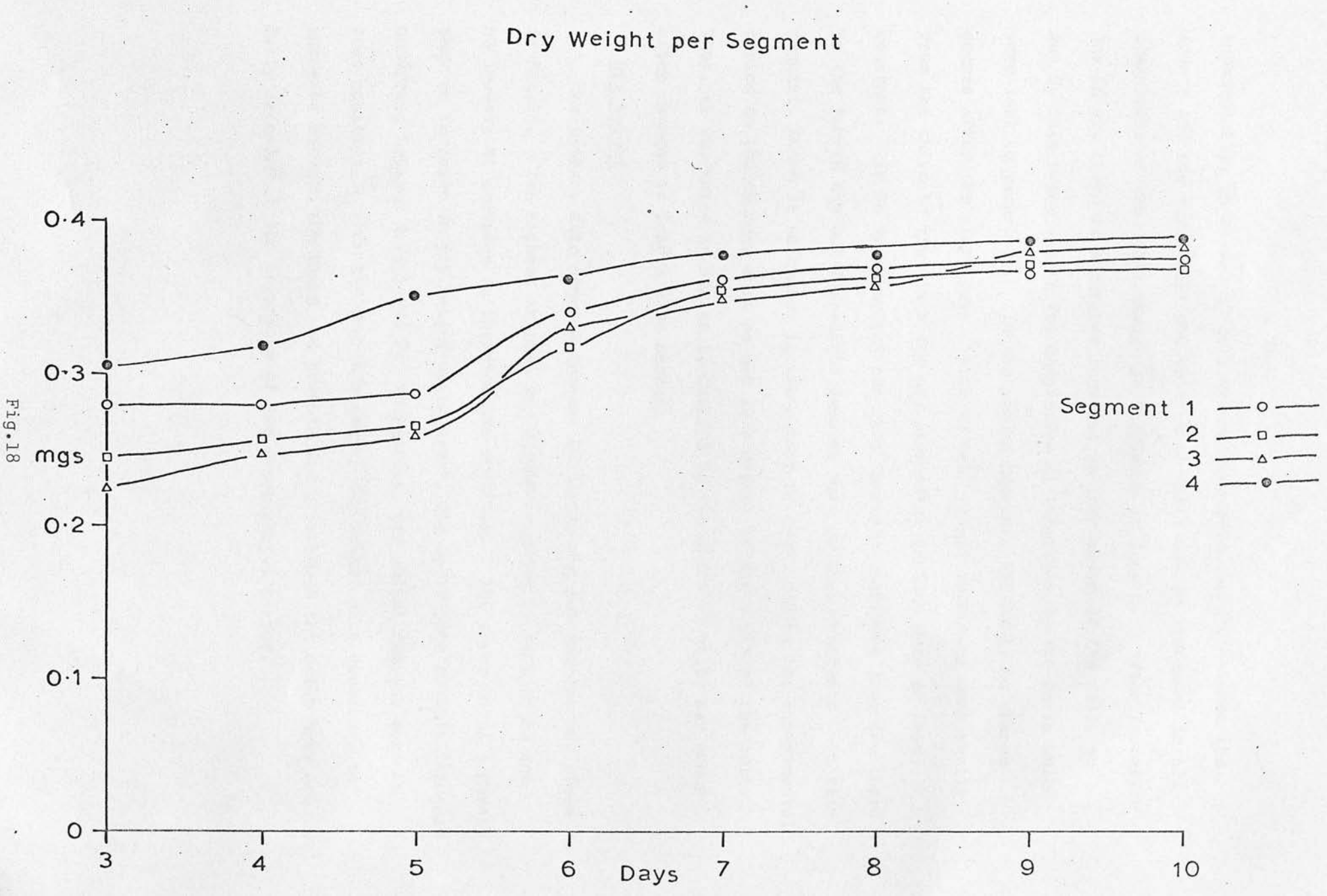

Dry Weight per Segment
0·4
0·3
0·2
0·1
0
mgs
3
4
5
6
7
8
9
10
Days
Segment 1
2
3
4

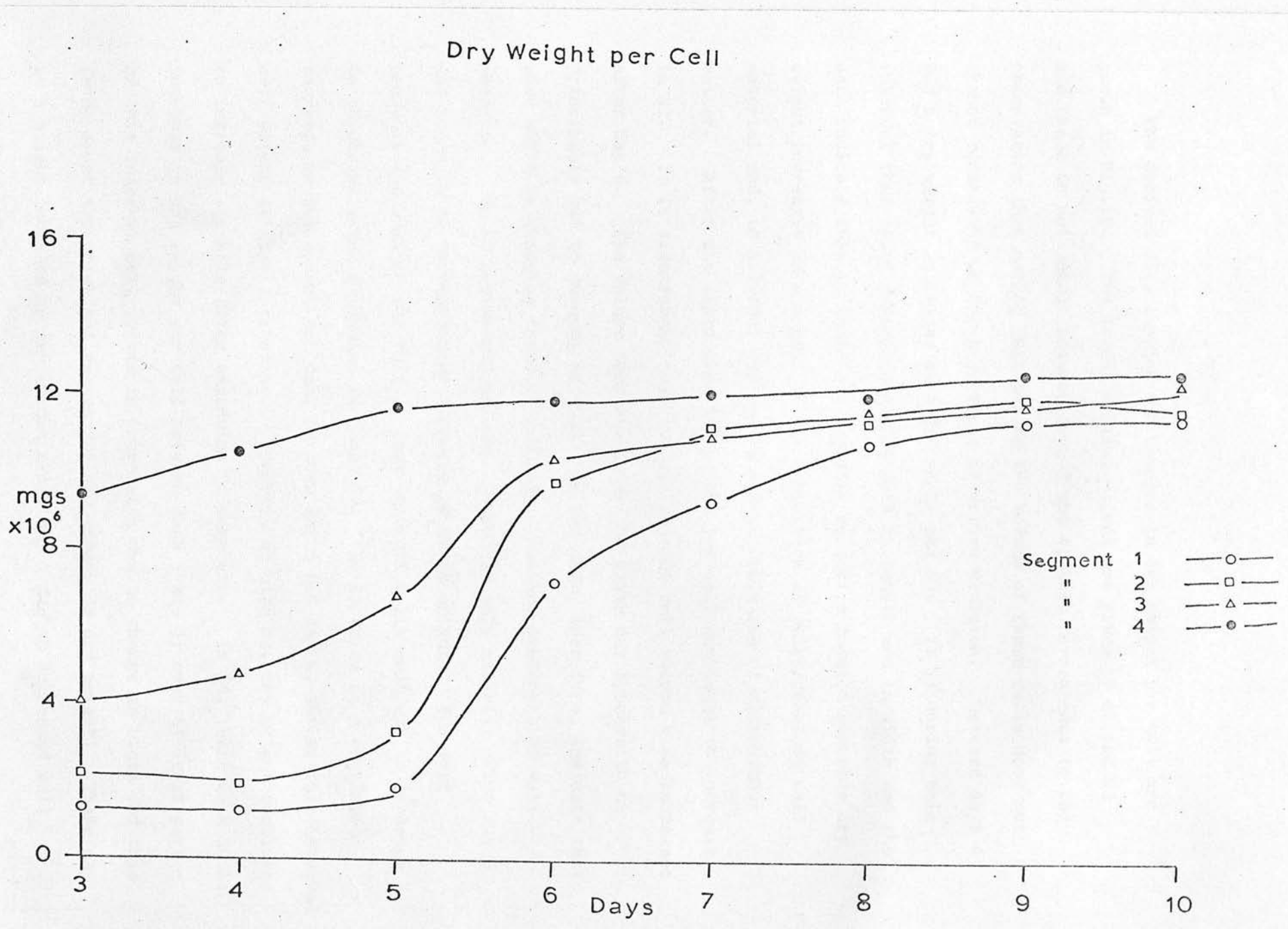
Fig. 18

Subsequently, in S1 and S2 cell volume increases sharply between the fourth and the sixth days and this is no doubt due to increases in all dimensions of the cells though particularly in length. After the sixth day in S1, there is a further increase in the volume of the cells to day 9, this being due to the completion of expansion by the cells which were last to vacuolate. In the second segment, virtually no change occurs after the sixth day. With S3 cell volume increases continually from the third to the sixth day and thereafter remains more or less constant. In S4 fresh weight per cell increases markedly from the third to the fourth day but thereafter remains more or less constant. In this segment, there is no change in the number of cells during the experimental period so the changes must be due to a change in the width of the cells. Thus, as was noted in Series 1, changes in the width of cells may occur after changes in length have ceased.

Dry Weight

The primary data showing changes in dry weight per segment are given in Fig.18. The highest initial dry weight is given by S4 with S2 and S3 lowest; S1 occupies an intermediate position. The three basal segments show an increase in dry weight up to Day 7, the major part of this increase occurring between days 5 and 7; thereafter, dry weight remains more or less constant. With the fourth segment, dry weight also continues to increase between the third and seventh days, although the cells here are fully extended at the beginning of the experimental period.

Fig.19


Dry Weight per Cell
16
12
mgs
x10⁶
8
4
0
3
4
5
6
Days
7
8
9
10
Segment 1
" 2
" 3
" 4

The derived data showing the changes in dry weight per cell are shown in Fig.19. The lowest initial values are given by S1 and S2 and these do not change between days 3 and 4; this corresponds to the observation that during this period the volume of these cells does not change consequent on the occurrence of active division. Between days 4 and 6 dry weight increases markedly in S1 and S2. It is during this interval that rapid expansion occurs both in length and in width and the data indicate that in these two segments as cell expansion proceeds dry weight increases as a result of the deposition of polysaccharide wall material and, to a lesser degree, to the accumulation of nitrogenous matter. After the sixth day, dry weight per cell continues to increase in S1. It is significant that in this segment cell volume also increases after day 6. The volume increase after the sixth day appears to be principally due to changes in width and the data, therefore, indicate that when width is changing there is probably a further synthesis of wall material. In S2 dry weight per cell increases only slightly after day 6 and there is no corresponding increase in fresh weight. The most probable explanation for this is that the cell walls continue to increase in thickness after expansion is complete. In S3 there is a continuous increase in dry weight per cell between day 3 and day 6, during this interval cell volume is also increasing. However, as with S2, dry weight continues to increase slightly after expansion is complete. In S4, there is a small increase in dry weight per cell between days 3 and 5; over at least part of this interval cell volume is increasing due to change in width and this is no doubt the principal reason for the change in dry weight. There may be a slight increase in dry weight after day 5 due to increased wall thickness.

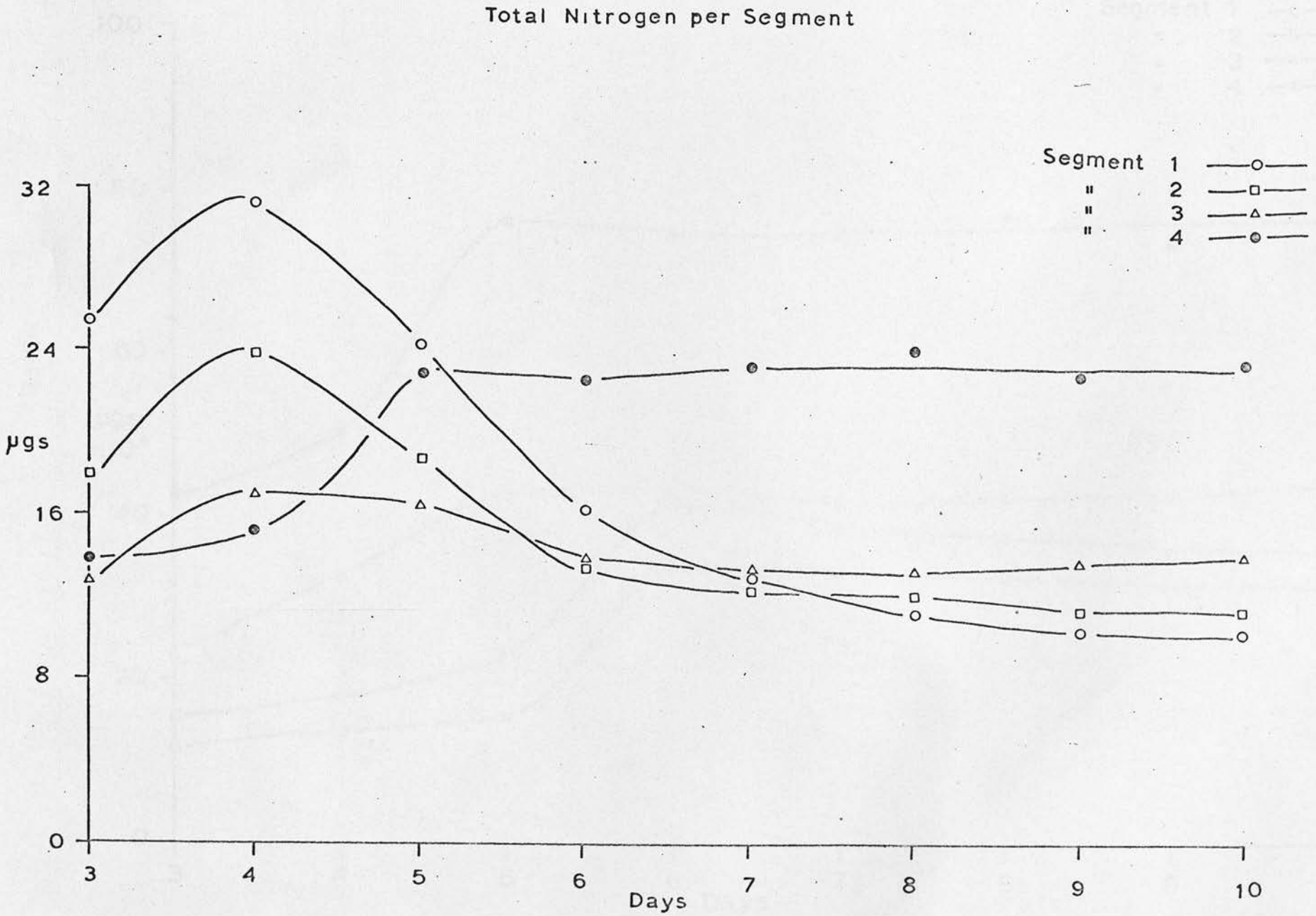

Total Nitrogen per Segment
Segment 1
" 2
" 3
" 4
32
24
16
8
0
µgs
3
4
5
6
7
8
9
10
Days

Fig.20

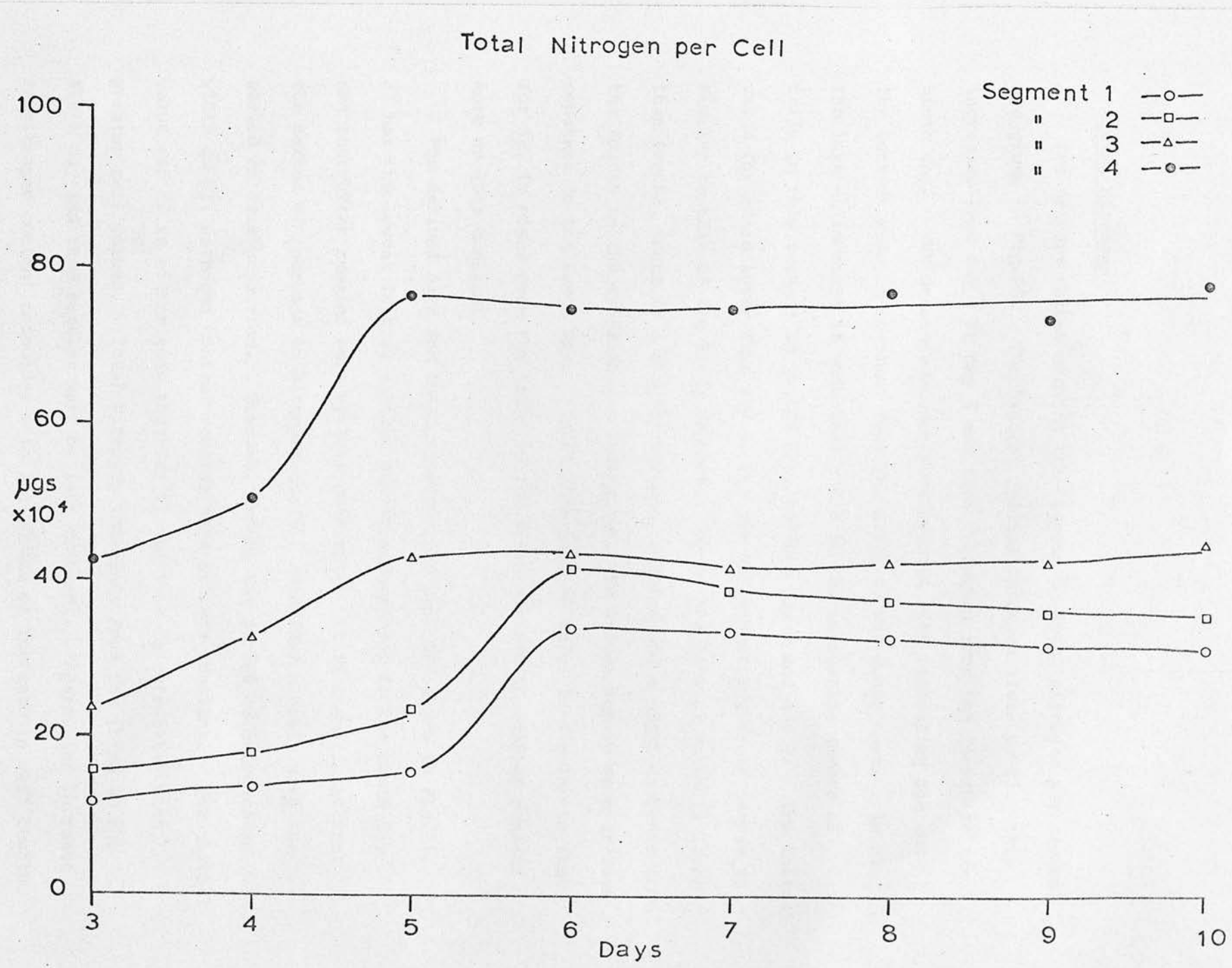

Total Nitrogen per Cell
Segment 1
" 2
" 3
" 4
100
80
60
40
20
0
µgs
x10⁴
3
4
5
6
7
8
9
10
Days

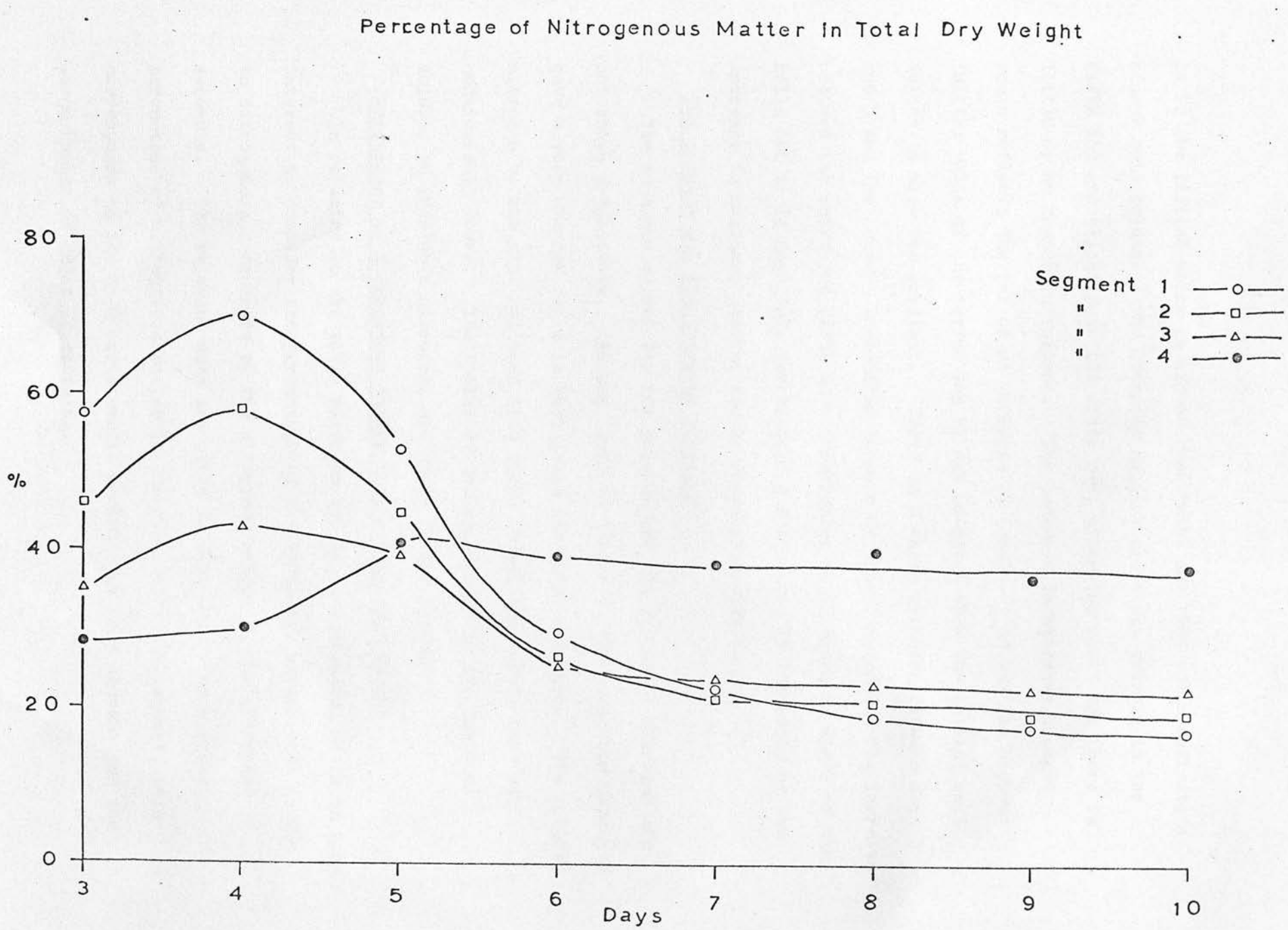
Fig.21

Total Nitrogen

The primary values showing the changes in total nitrogen per segment are given in Fig.20. The highest initial value is given by S1; this increases from day 3 to day 4 and then decreases from the fourth to the ninth day. The decrease becomes more gradual with increasing age and the content remains constant from the ninth to the tenth days. In S1 the initial increase is undoubtedly due to the increasing number of cells in this segment in the 24 hrs. between day 3 and day 4. The initial value for S2 is lower than for S1 but the subsequent course of change is similar to that of the first segment. In S3 the initial value is lower than for S2, there is a slight increase to day 4 and a slight decrease from the fourth to the sixth day. Thereafter, the values remain more or less constant to the tenth day. In S4, the initial value is similar to that for S3, it rises from the third to the fifth day and thereafter remains more or less constant.

The derived data for total nitrogen per cell are given in Fig.21. S1 has the lowest initial content but this increases to the sixth day and thereafter remains more or less constant. It is significant that the period of increase in nitrogen content coincides closely with the period of cell expansion. However, during the period of increase in width in S1, nitrogen content remains more or less constant. The initial value for S2 is higher than that for S1 and this is correlated with greater cell volume. Total nitrogen increases from the third to the sixth day and then remains more or less constant. Again, the increase in nitrogen content coincides with the period of increase in cell length.

Fig.22


Percentage of Nitrogenous Matter in Total Dry Weight
80
60
40
20
0
%
Segment 1
" 2
" 3
" 4
3
4
5
6
7
8
9
10
Days

In S3 the initial value is higher than in S2 and this again reflects a higher cell volume. The nitrogen content increases sharply to the fifth day and slightly to the sixth day, after the sixth day there is little or no change in content. The increase in nitrogen content again reflects the period of increase in length. S4 has the highest initial value of the series and it may be noted that the initial cell volume is also the greatest. There is a sharp increase between days 3 and 5 and the content thereafter is more or less constant. The increase between the third and fifth days accompanies an increase in width of the cells but it is doubtful, for reasons given below, if the whole of the increase in nitrogen content can be correlated with this.

TCA soluble and TCA insoluble nitrogen

The separate values for TCA soluble and TCA insoluble nitrogen are not shown graphically. As was found in Series 1, the insoluble nitrogen gave a very similar curve to that found for total nitrogen. The soluble nitrogen values also followed this curve though the values here were considerably lower. The tables of values, including the ratio of soluble to insoluble nitrogen, are given in the Appendix.

Percentage of Nitrogenous matter in the Total Dry Weight

In relation to the total nitrogen and dry weight data, it is of some interest to consider the percentage of the total dry weight that is due to nitrogenous components at the different stages of the different segments. The relevant data are given in Fig. 22. The highest percentage of nitrogenous matter is found in the first segment, this corresponds to the high meristematic activity of this segment and the large number of cells it contains.

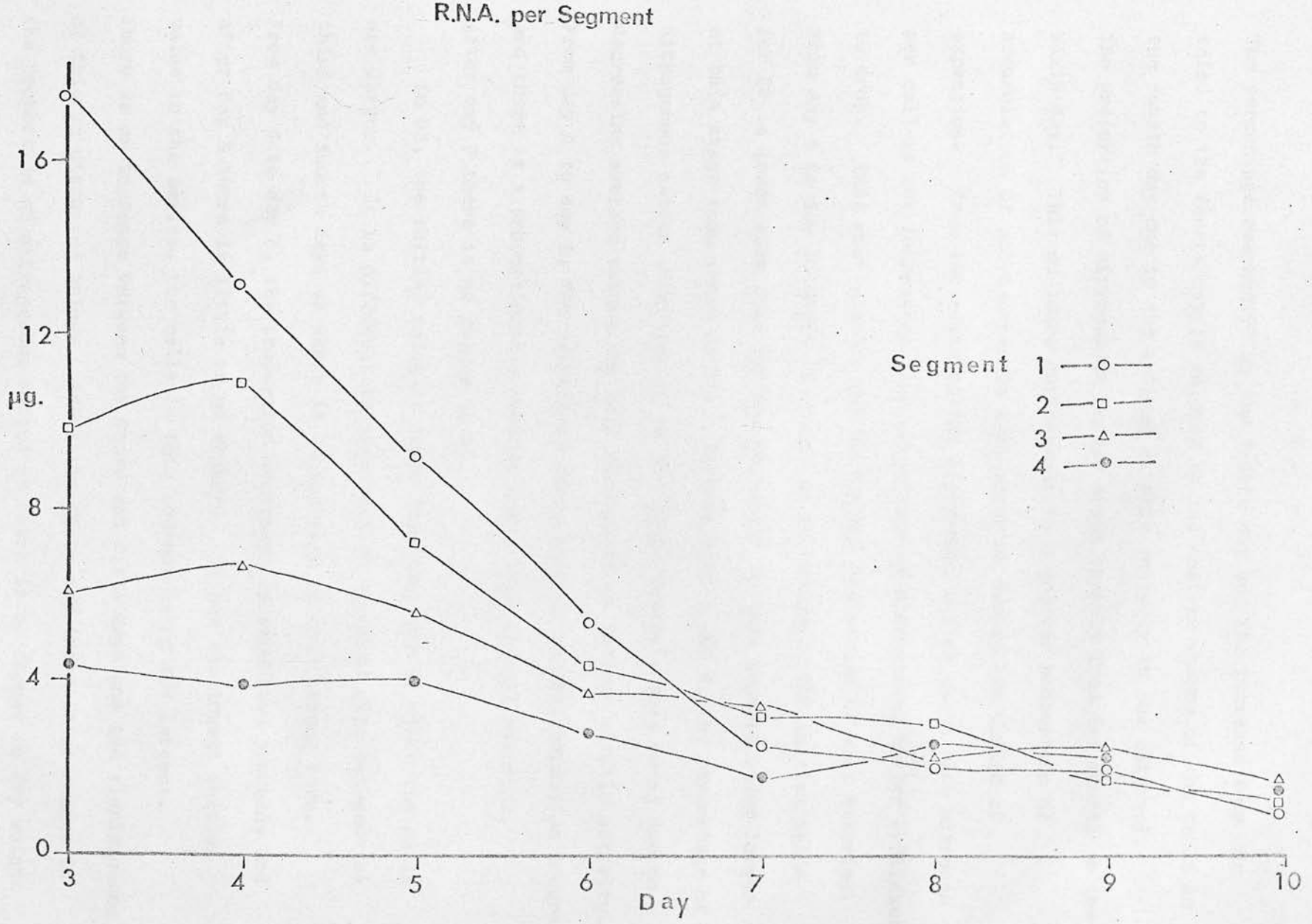


Fig.23

The percentage reaches 70 on the fourth day and the increase from the third to the fourth day is related to the smaller volume of the cells on the fourth day due to the intense mitotic activity in the interval. The proportion of nitrogenous matter drops sharply from the fourth to the sixth day. This no doubt corresponds to a greater proportion of accumulation of polysaccharide wall material during the period of expansion. From the sixth to the eighthday, during which time nitrogen per cell is not increasing, the percentage of nitrogenous matter continues to drop. This must also be due to further deposition of wall material. From day 8 to day 10 there is little or no change. The initial value for S2 is lower than that for S1, the cells in this segment being larger at this stage than those of S1. Between days 3 and 4, the percentage of nitrogenous matter increases as in the first segment, this being due to decreasing average volume per cell consequent on intense mitotic activity. From day 4 to day 6, the percentage drops sharply as cell expansion occurs and there is a proportionally greater deposition of wall material. After day 7 there is no change in S2.

In S3, the initial value is lower than that for S2 since the cells are larger. It is difficult to interpret an apparent rise between the third and fourth days as there is no increase in cell number here. From day 4 to day 6, the proportion decreases as expansion proceeds and after day 6 there is little or no change. S4 has the lowest initial value in the series, the cells in this segment being the largest. There is an increase between the third and fifth days and the significance of this is discussed below. After the fifth day there is no change in the proportion of nitrogenous matter as there is no change in dry weight or in nitrogen content.

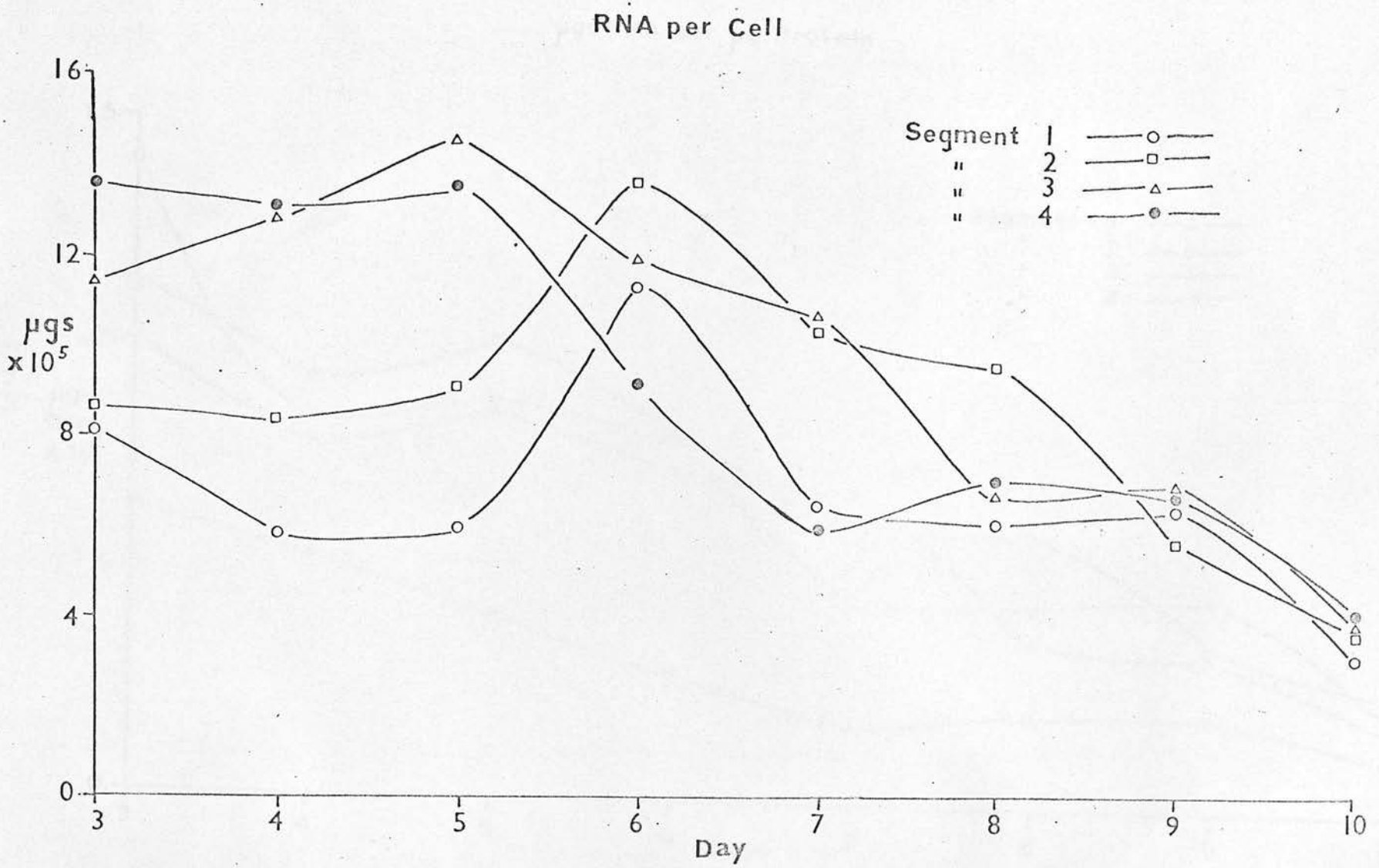


Fig.24

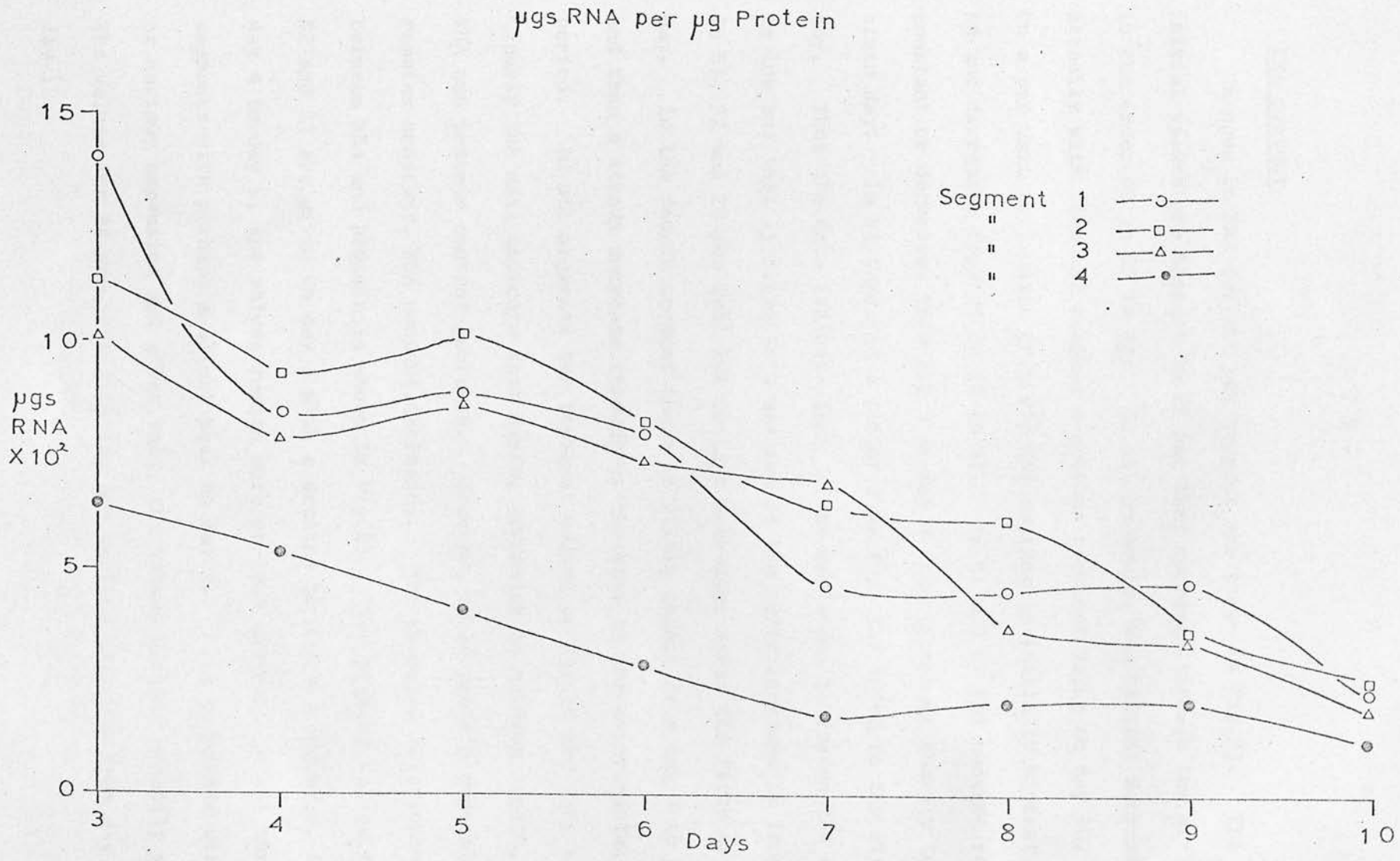

µgs RNA per µg Protein
15
10
5
0
µgs
RNA
X10²
3
4
5
6
7
8
9
10
Days
Segment 1
" 2
" 3
" 4

Fig.25

RNA content

Changes in RNA content per segment are shown in Fig.23. The initial values are highest in S1 and they decrease through the series in the order S2 to S3 to S4. In all segments, RNA content decreases steadily with time and reaches a similar terminal value on day 10. On a per unit cell basis (Fig.24) RNA content is initially highest in S4 and decreases from S3 to S2 to S1. In S1 and S2 RNA content remains constant or decreases from day 3 to day 4 then increases sharply to the sixth day. In S3 there is a slight rise from the third to the fifth day. Thus the data indicate that in the meristematic region RNA content is low but that it rises to a maximum as the cells increase in length. In S1, S2 and S3 per cell RNA content decreases after the fifth or sixth day. In the fourth segment there is little change from day 3 to day 5 and then a steady decrease throughout the rest of the experimental period. In all segments the terminal values on day 10 are very similar. Clearly the data indicate that during expansion to maximum length, both RNA and protein content increase. However, while protein content then remains constant, RNA content decreases. The changing relationship between RNA and protein is shown in Fig.25. The highest values for S1, S2 and S3 are given on day 3 with a decline to day 4. However, from day 4 to day 6, the values remain more or less constant in all three segments with perhaps a slight peak on day 5. This coincides with the period of maximum expansion and after this, the values decline steadily with time. The values for S4 are initially low and decline steadily from day 3 to day 10.

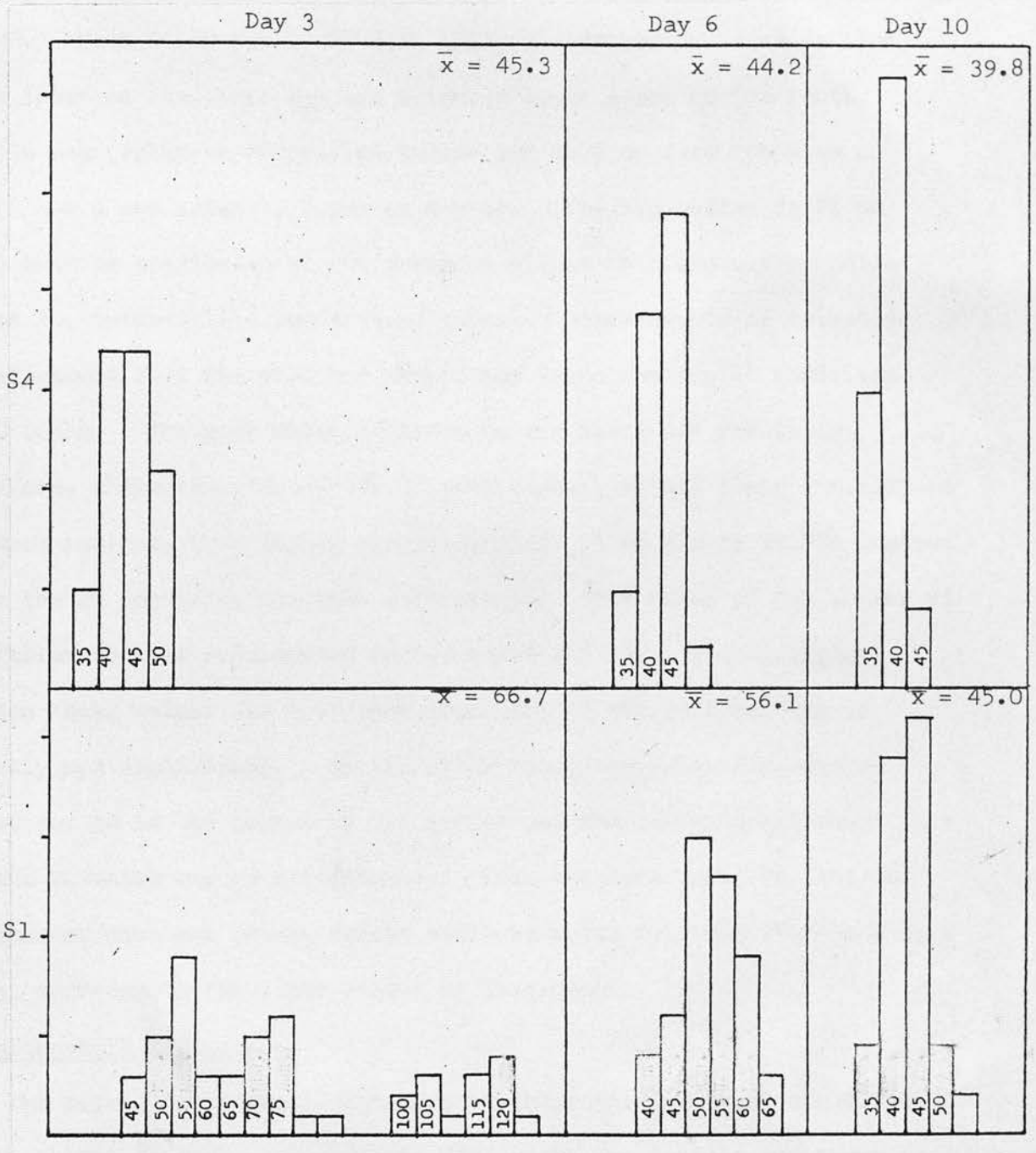


Fig.26 Histogram showing relative adsorption values for D.N.A. in the nuclei of cells in S1 and S4 on days 3, 6 and 10. The divisions on the left-hand side of the diagram represent 10% of the population. The mean values ($\bar{x}$) are also shown.

DNA content

Limited data on DNA contents of nuclei in S1 and S4 are shown in Fig.26. With S1 on day 3 the mean relative absorption value is high, it is lower on the sixth day and slightly lower again on the tenth. With S4 mean relative absorption values are more or less the same on days 3 and 6 and slightly lower on day 10. The high value in S1 on day 3 must be attributed to the presence within this segment of cells in the 2C, intermediate and 4C conditions. Measurements of telophase nuclei showed that the relative absorption value for the 2C condition is 50 to 55. The mean value recorded on the sixth day for S1 is, therefore, characteristic of the 2C condition. Within their limitations the data indicate that during expansion there is no change in DNA content after the 2C condition has been established. The value on day 10 for S1 is similar to that recorded on days 3 and 6 for S4. The difference between these values and that characteristic of the 2C condition is probably not significant. On the other hand, the value recorded on day 10 for S4 is the lowest of the series and the difference between this and the 2C value may be significant. Thus the data indicate that the DNA content does not change during early maturity but that there may be a slight decrease in the later stages of development.

Chlorophyll content

The primary data showing changes in chlorophyll content are given in Fig.27. The initial values for S1, S2 and S3 are similar and there is only a small increase in these segments until day 6 or 7. With S3, on the sixth day chlorophyll content rises slowly and continues to rise to day 10. With S1 and S2 this increase starts on day 7 and is still continuing on day 10.

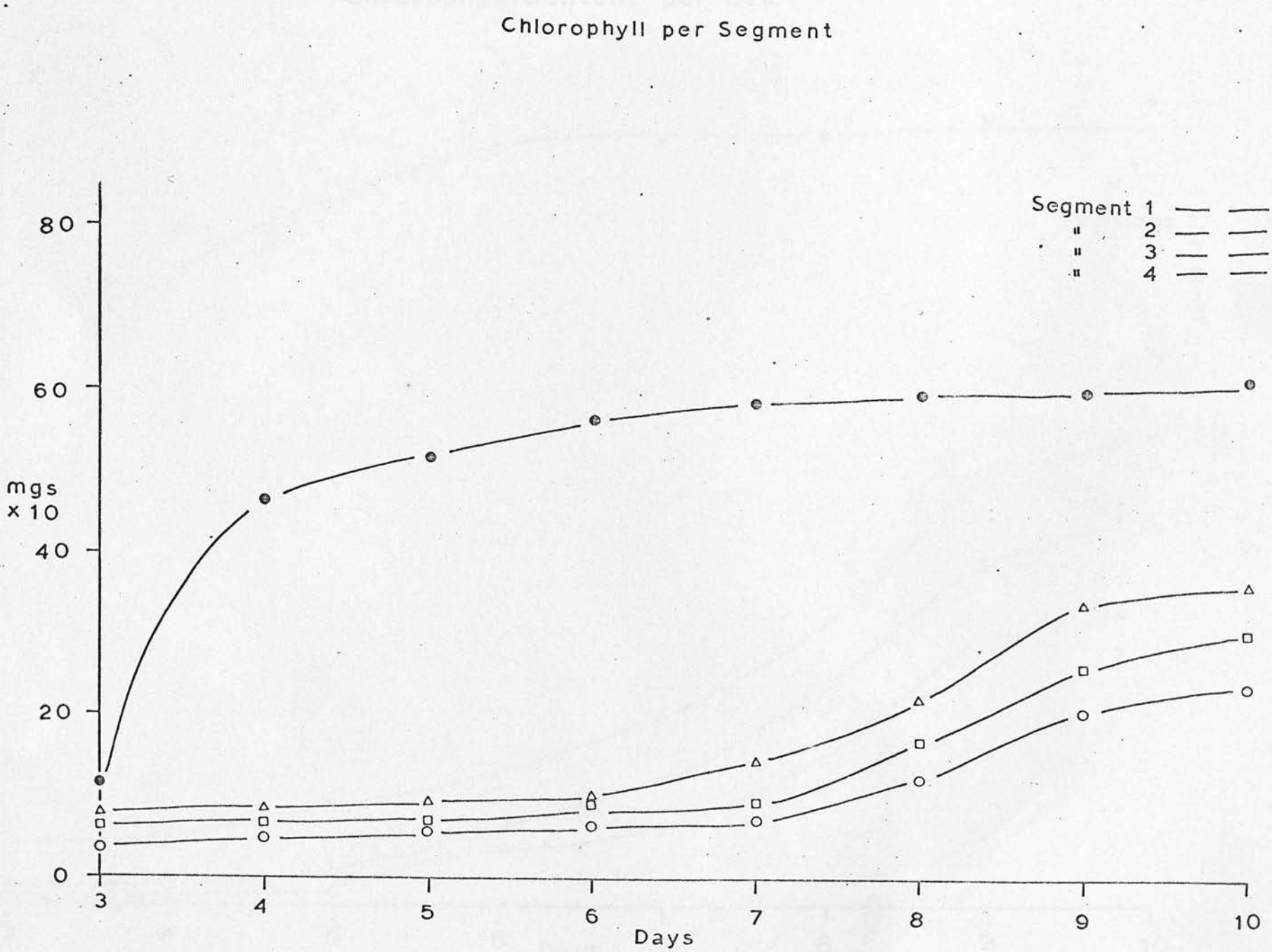

Chlorophyll per Segment
Segment 1
" 2
" 3
" 4
80
60
40
20
0
mgs
x 10
3
4
5
6
7
8
9
10
Days

Fig.27

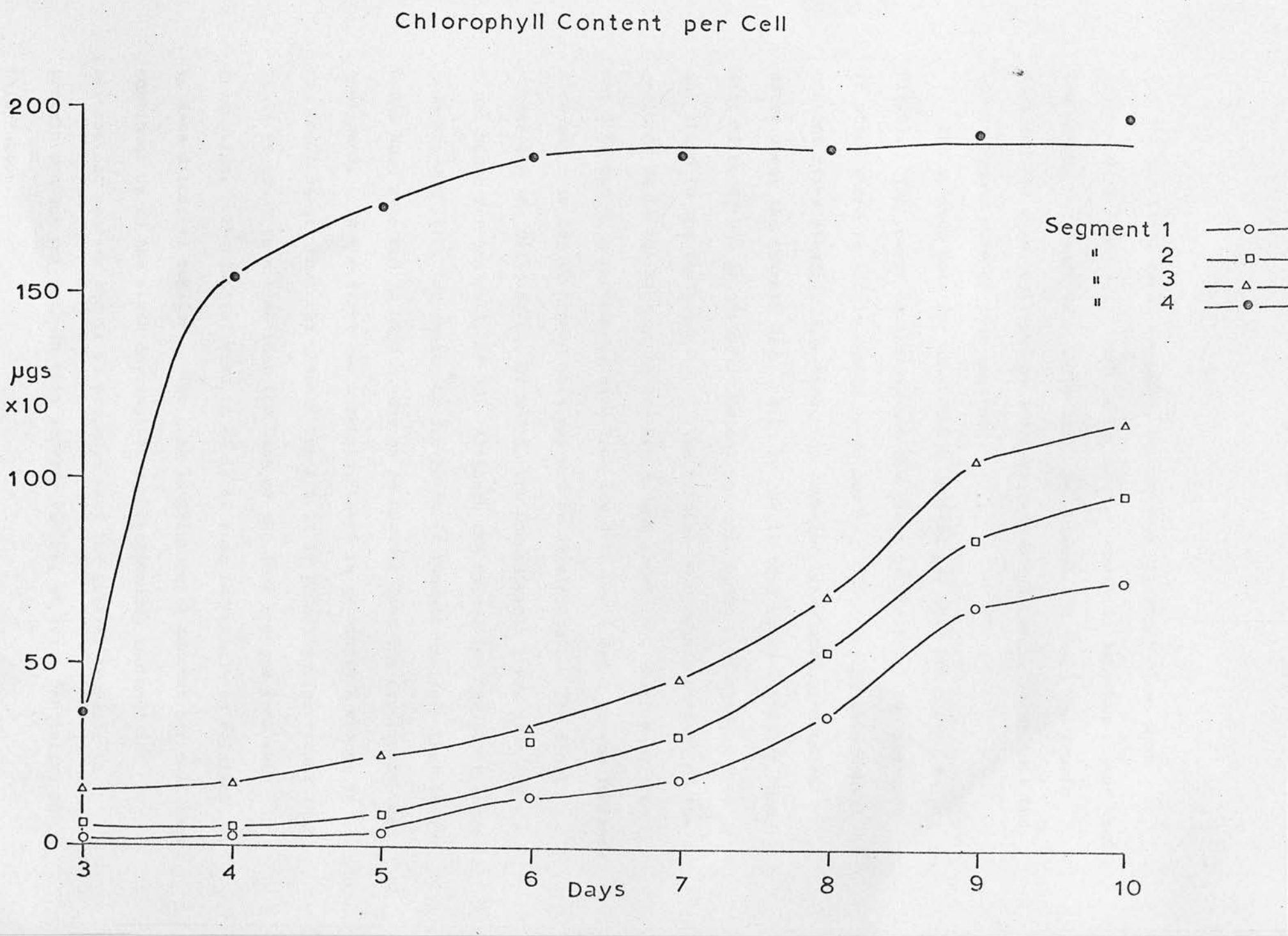

Chlorophyll Content per Cell
200
150
µgs
x10
100
50
0
3
4
5
6
Days
7
8
9
10
Segment 1
" 2
" 3
" 4

Fig.28

For all the first three segments, the rate of increment slows down between days 9 and 10. With S4 the initial value is somewhat higher than the others but there is a sharp increase between the third and fourth days and the rise continues at a slower rate until day 7, after this the values remain more or less constant.

The derived data for chlorophyll content per cell are shown in Fig.28. The lowest initial values are given by the first two segments in which there is little change up to day 5. After day 5, chlorophyll content rises steadily with time, the increase being more pronounced after about the seventh day. With S3 the initial value is higher than with S1 or S2 and chlorophyll content per cell rises continuously from the third to the tenth day. The initial chlorophyll content of the cells of S4 is the highest in the series and there is a sharp rise from day 3 to day 4, a pronounced rise from day 4 to day 6 and a slower increase from day 6 to day 10 though this may not be significant. The final values for the cells of S1, S2 and S3 are considerably lower than the final value for the cells of S4. Although the values for the first three segments were still increasing on day 10 it is thought unlikely that they would have attained as high a value as S4 however long the experiment was continued. In the first two segments, there is evidently a change in the position at about the seventh day and it is possibly significant that it is at about this time that the base of the leaf emerges from the coleoptile. The initial rise in S4 is of some importance in relation to data discussed earlier. The rise in chlorophyll content per cell that continues up to the sixth day implies a corresponding increase in chloroplast protein and it is probable that the initial increase in protein content per cell in this segment is due, at least partially, to this factor.

Further, the increase in the percentage of protein in the total dry weight over this period is again no doubt due to the relatively intense development of chloroplasts. Although in S4 increase in chlorophyll is accompanied by an increase in protein content, this is not the case with S1, S2 and S3 after day 6. However, until day 6 or 7 these segments are shielded by the coleoptile and the subsequent increase in chlorophyll could possibly be accommodated in previously developed but more or less colourless chloroplasts.

Discussion

The array of data presented above indicates certain general characteristics of expansion process in the leaf which, to a certain extent, confirm and amplify the results reported in Series 1. Although here in S1, S2 and S3 expansion apparently proceeds over 48 hrs., this does not mean that the process in the individual cells also occupies this time. As indicated earlier, the average in the individual segments is determined by the displacement of cells along the leaf and the situation is dominated by the basal segment. The data for cell number per segment (Fig. 15) are not inconsistent with the conclusion reached in Series 1 that the process of expansion in length of individual cells occupies 24 hrs. The present series of data show, however, that expansion occurs laterally as well as longitudinally. It is probable that expansion in the initial phase involves both axes but it is clear from the data for the first segment that after expansion in length has ceased, expansion in width may continue. The present series of data confirm the conclusion that expansion is accompanied by changes in dry weight and that these changes are primarily due to accumulation of polysaccharide material in the cell walls.

It is evident that this growth of the wall occurs both in connection with lateral and longitudinal growth. Part of the increase in dry weight during cell expansion is due to nitrogenous constituents, as with other meristematic systems examined, the proportion of nitrogenous constituents is relatively high in the meristematic cells and decreases as these cells expand as the result of relatively more rapid synthesis of carbohydrates. It is of some interest that while the phase of expansion in length and width is accompanied by an increase in nitrogen, that of increase in width alone in S1, S2 and S3 apparently is not. It is further of interest that when expansion has ceased there is no decrease in total nitrogen.

It was noted in Series 1 that the cells of the leaf appeared to pass through the same quantitative stages as they matured. This is evidently not the case in Series 2, here the cells that are derived from the expansion of the intercalary meristem differ at maturity from those derived while the meristem was still active. This is particularly well shown in the different terminal total nitrogen contents found for segments 1, 2 and 3 and for S4. The latter had approximately twice as much nitrogen on day 10 as S1. A similar though not so pronounced difference is shown by the terminal values for chlorophyll content.

c) Metabolic Characteristics

The changes in the cellular pattern described in the last section imply corresponding changes in metabolic characteristics. This was investigated through observations on respiration and through the activities of three enzyme systems: in Series 2, measurements of photosynthetic rate were also made. Respiration gives a general measure of metabolic activity and the activities of particular enzymes gives an indication of the general catalytic properties of cell proteins.

Series 1

The graphs are presented in a similar manner to those for quantitative characteristics. Results on a unit segment basis are shown in Fig.29, on a unit cell basis in Fig.30 and on a unit protein basis in Fig.31. Each of the values shown is the mean of three separate determinations and the original data are given in tabular form in the Appendix.

Respiration

At 72 hrs., respiration per segment (Fig.29) is highest in S1 and decreases sharply from here to S4 and then slightly to the apex. At 96 hrs., respiration is highest in the first two segments, decreases sharply from here to S4 and then very slightly to the apex. On a unit cell basis (Fig.30) at 72 hrs. respiration is lowest in S1 and rises sharply from here to S4; it remains more or less constant to S6 but declines at the apex. At 96 hrs. respiration per cell is again lowest in the basal segment, rises sharply to S3 and then remains more or less constant throughout the rest of the leaf. In terms of unit protein (Fig.31), respiration is low in the base of the 72 hr. leaf, rises to S2 and S3 and then declines with each successive segment to the apex.

At 96 hrs., the picture is very similar with a rise to S3 and then a progressive decline to the apex.

It is evident that specific respiratory activity is low in the meristem and that it increases with increasing vacuolation. The absolute increase per cell is no doubt a reflection of the increasing specific activity and of the increasing protein content of the cells. The decrease in the specific activity between the third segment and the apex does not necessarily mean that the specific activity of the cytoplasm is decreasing with increasing distance from the base and, therefore, with age. Indeed, the per cell data suggest that the respiratory activity of the cytoplasm is, in fact, remaining constant. It may be recalled that in both sets of leaves chlorophyll content increases from the base to the apex of the leaf. This implies an increase in chloroplast protein as the cell develops, and the apparent decrease in respiratory activity, when the data are expressed on a unit protein basis, may reflect an increasing proportion of chloroplast protein, which is inert with respect to respiration.

Invertase

Invertase activity per segment (Fig.29) is low at the base of the 72 hr. leaf and increases sharply up to S3; this is followed by a decline to the apical segment. At 96 hrs., the position is very similar - a low value in S1 followed by a rise to S3 and then a fall to S7. This decline continues to the apex but is less pronounced between S7 and S11. When the values for invertase are expressed on a unit cell basis (Fig.30) they are low in S1 of both leaves and rise from this to S4.

There is then a steady decline to S7; subsequent to S7 in the 96 hr. leaf the values remain more or less constant to S11 and then decline again to the apex. On a unit protein basis (Fig.31) at 72 hrs. the picture is again very similar to that found per segment and per cell with a rise from S1 to S3 and then a decline to the apex. At 96 hrs. the peak is at S4 followed by a decline to the apex.

It is evident that specific activity per unit protein is low in the meristematic zone and increases from this as expansion proceeds to S3 and S4. This trend is apparent in both sets of leaves. Between the third and the apical segments at 72 hrs. activity drops sharply, it also drops sharply in the 96 hr. leaf between S4 and S7 but thereafter declines gently to the apex. The values expressed on a unit cell basis show that activity is low in the meristematic zone and rises to a peak in the third and fourth segments. This rise in activity coincides with the extension of the cells. With both sets of leaves, the increase from the basal to the fourth segment is no doubt referable to increasing specific activity and to increasing protein content. Activity per cell decreases from the fourth to the seventh segments in both leaves; in the 96 hr. leaf this is followed by a constant value from the seventh to the eleventh segments and then a further drop to the apex. These changes in both sets of leaves undoubtedly reflect corresponding changes in specific activity. They suggest that once extension is complete the specific activity of invertase decreases with increasing distance from the base and, therefore, with age.

Phosphatase activity

The primary values for the individual segments are shown in Fig.29. At 72 hrs. it is evident that activity is highest in the basal segment and declines steadily from this to the apex. At 96 hrs. there is possibly a slight increase from S1 to S2 and a slight decrease from S2 to S3, but from here there is little change to the apex. It is possible that there is no significant change from the base to the apex.

On a per unit cell basis (Fig.30) phosphatase activity is lowest in S1 at 72 hrs. and then rises sharply to the third segment thereafter remaining more or less constant. At 96 hrs., activity is again lowest in the basal segment, rises steadily to S5 and remains more or less constant to the apex. On a unit protein basis (Fig.31) phosphatase activity appears to be relatively low in S1, rather higher in S2 and then decreases with progress along the leaf to the apex. At 96 hrs. activity is also low in S1, it increases gradually to S5 and then decreases gently to the apex. It may be noted that the value for phosphatase on a unit protein basis at 96 hrs. could be interpreted in terms of more or less constant activity in the different segments since the apparent increase from the base to S5 and the decline thereafter are only slight, although progressive.

In many respects, the results recorded in the phosphatase determinations are similar to those found with respiration. When expressed on a unit cell basis with both sets of data, there is a sharp increase from S1 to S4 or S5. When the results are expressed on a per unit protein basis there is again a slight initial rise followed by a subsequent slow decrease.

The data suggest that specific activity is low in the meristem, that it tends to increase with the process of expansion to a maximum value and then decreases. It may be emphasised that the decrease when extension is complete may not indicate a decreasing activity of the cytoplasm since with the development from the third to the fourth segment chloroplast protein may be increasing and the apparent drop may be due to an increasing proportion of protein being inert with regard to the phosphatase measured here. The rise in the per cell values from S1 to S3 or S4 no doubt reflects the influence of increasing mass of cytoplasmic protein. The relative constancy of the values between S3 and S4 and the apical segment suggests that the activity of the cytoplasm does not, in fact, decrease.

Protease

The primary values per segment (Fig.29) show that at 72 hrs. activity is highest at the base and declines with progress from this to S4. There is an apparent slight recovery to S5 and then a further fall to the apex. At 96 hrs. activity is highest in S1 and S2 decreases sharply to S5 and then rises slowly to S12 with a decline to the apex. On a unit cell basis (Fig.30) activity is lowest in S1 at 72 hrs. rises sharply to S3 with a slight decline to the apex thereafter. At 96 hrs. activity is again lowest in S1 but then increases steadily with increasing distance from the base of the leaf. On a unit protein basis (Fig.31) activity is high in S1 at 72 hrs. rises slightly from here to S3 and then declines sharply to S4. There is an apparent increase to S5 but a further decline to the apex thereafter. These fluctuations may be disguising a general trend of decreasing activity from the base to the apex. At 96 hrs. there is an apparent slight increase from the base to S2, a decrease from this to S3 and then a further increase to S4. After this the values remain more or less constant to the apex.

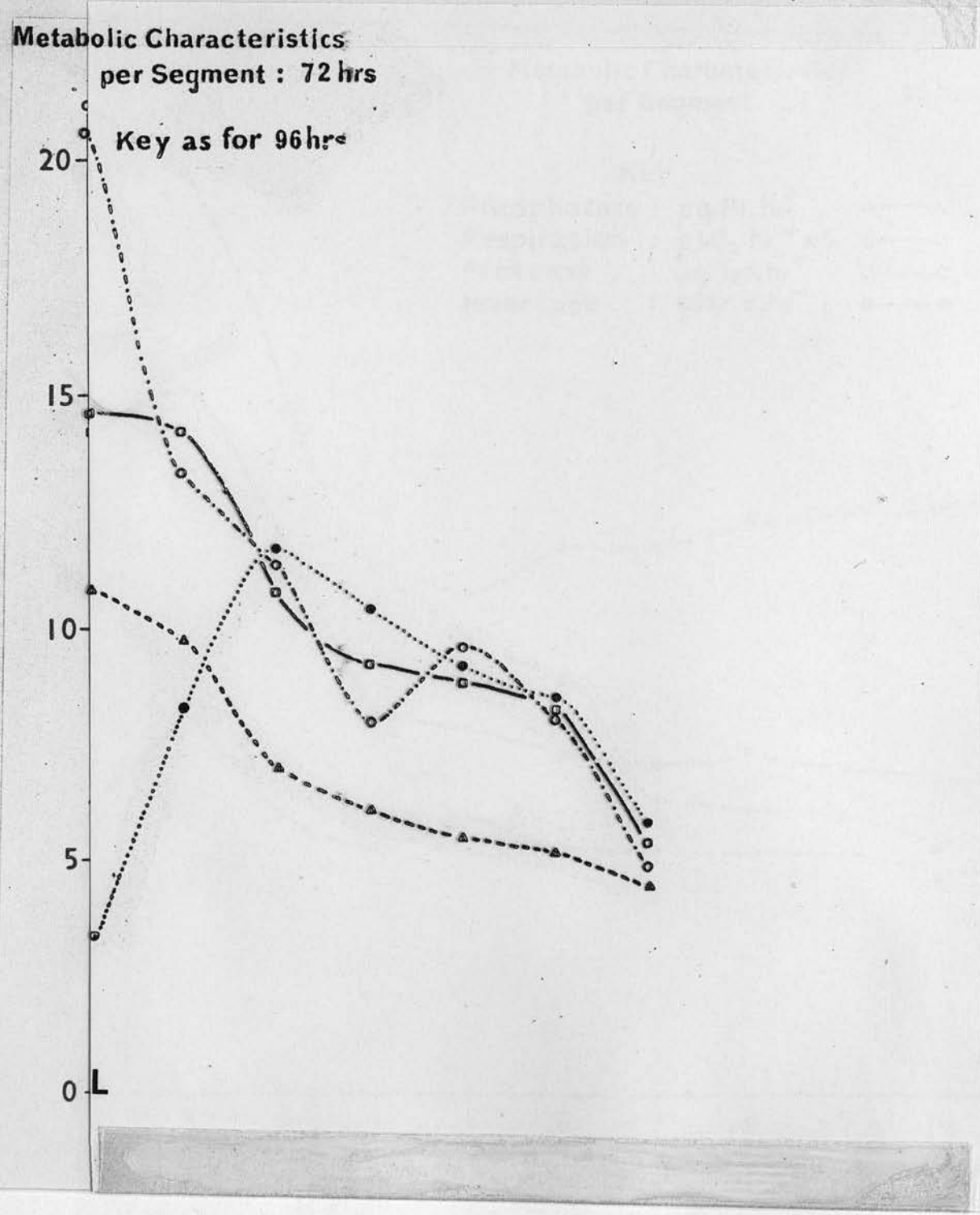

Metabolic Characteristics
per Segment : 72 hrs
Key as for 96 hrs
20
15
10
5
0
L

Fig. 29

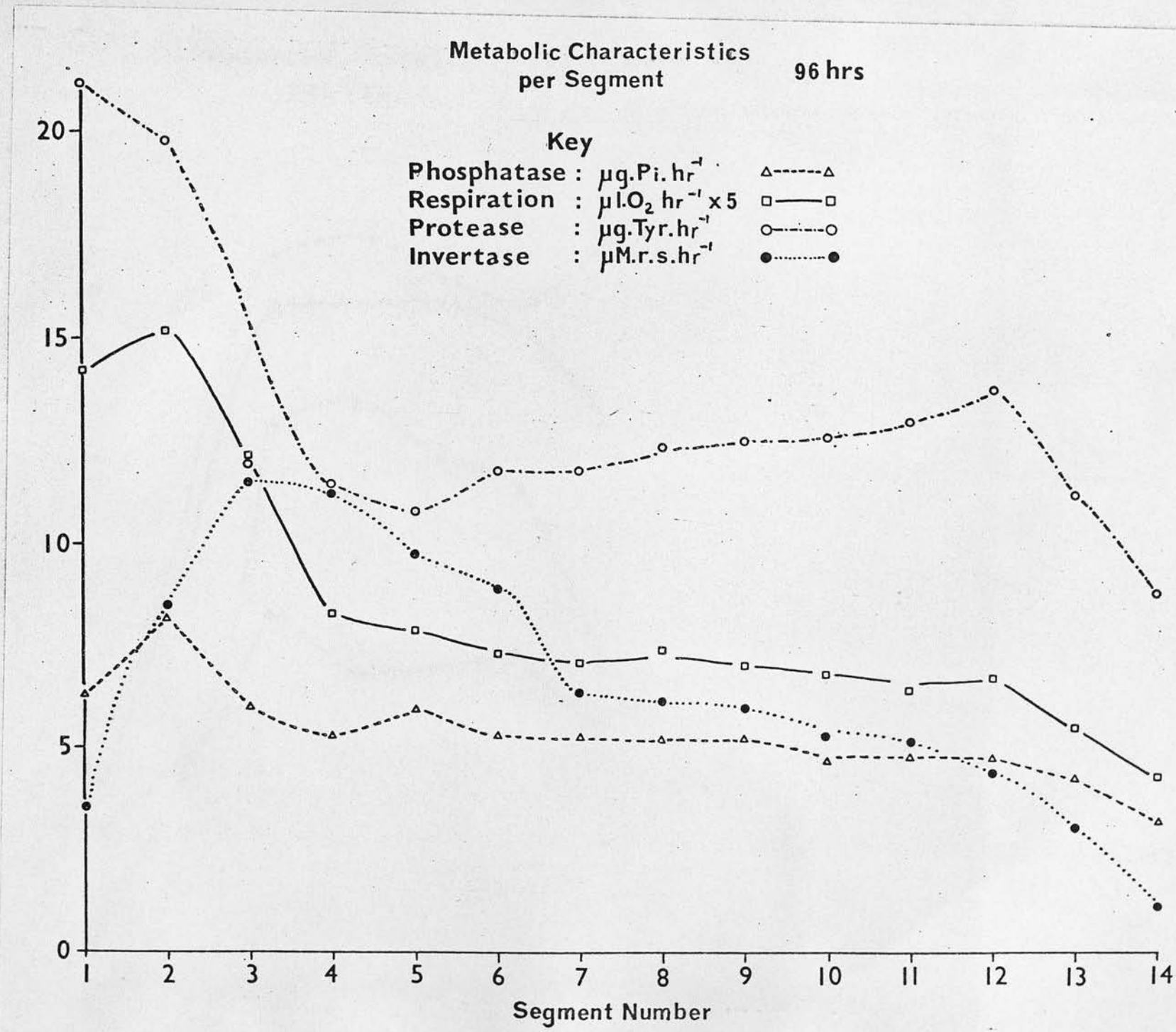

Metabolic Characteristics
per Segment
96 hrs
Key
Phosphatase : µg.Pi. hr⁻¹
Respiration : µl.O₂ hr⁻¹ x 5
Protease : µg.Tyr.hr⁻¹
Invertase : µM.r.s.hr⁻¹
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number

Fig.29

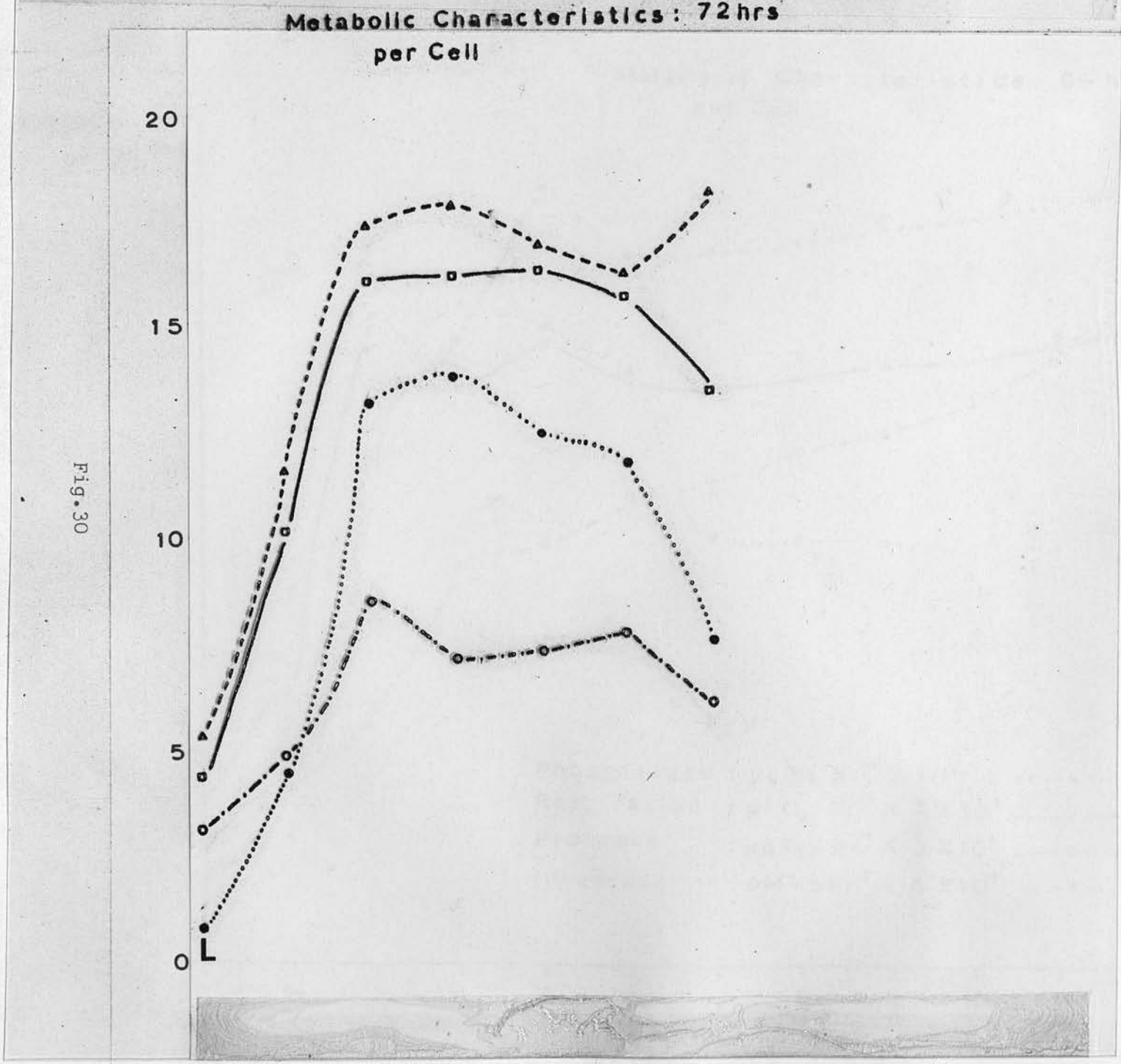


Fig. 30

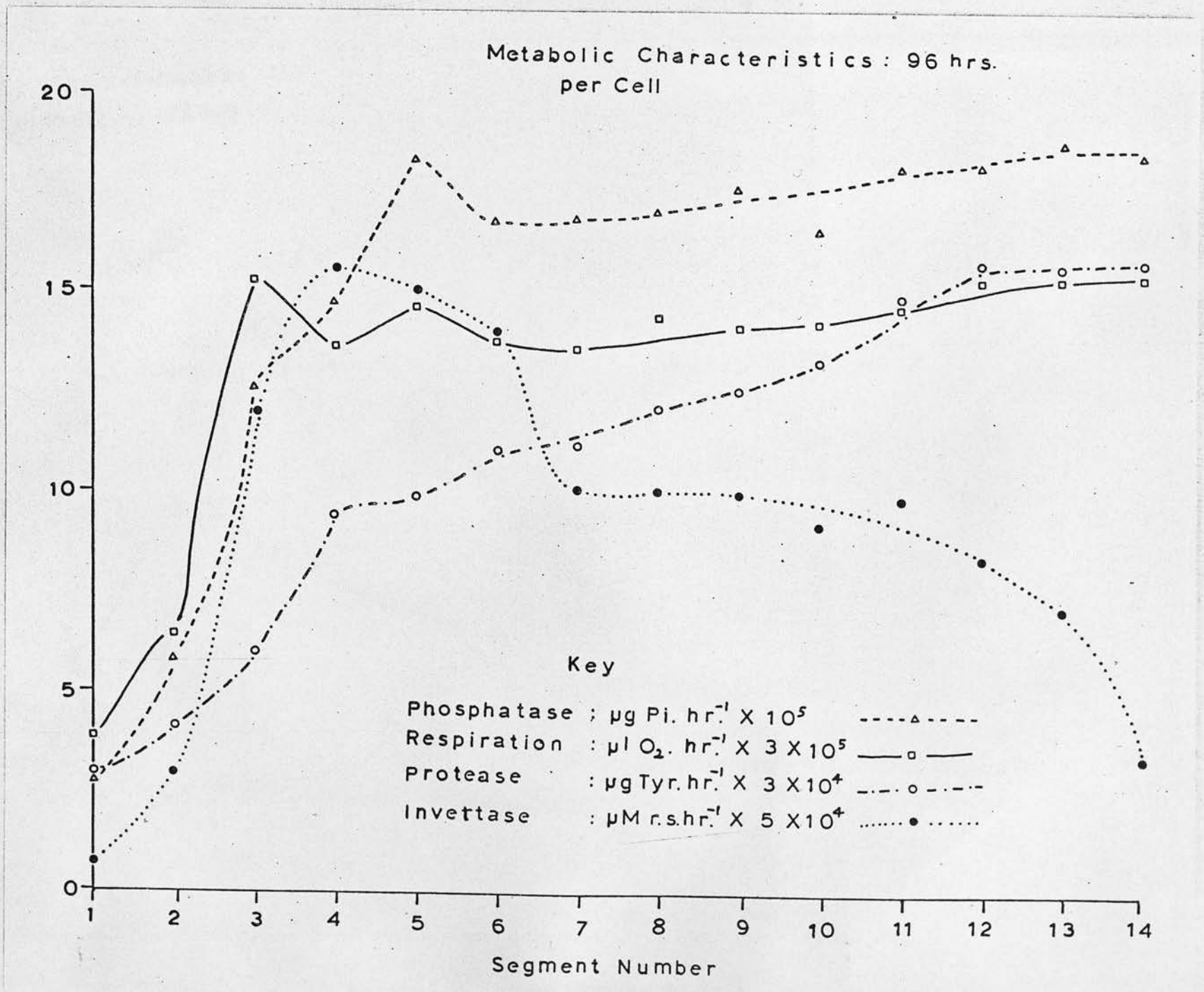

Metabolic Characteristics : 96 hrs.
per Cell
20
15
10
5
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number
Key
Phosphatase ; µg Pi. hr.⁻¹ X 10⁵
Respiration : µl O₂. hr.⁻¹ X 3 X 10⁵
Protease : µg Tyr. hr.⁻¹ X 3 X 10⁴
Invettase : µM r.s. hr.⁻¹ X 5 X 10⁴

Fig.30

Metabolic Characteristics
per µg Protein: 72 hrs

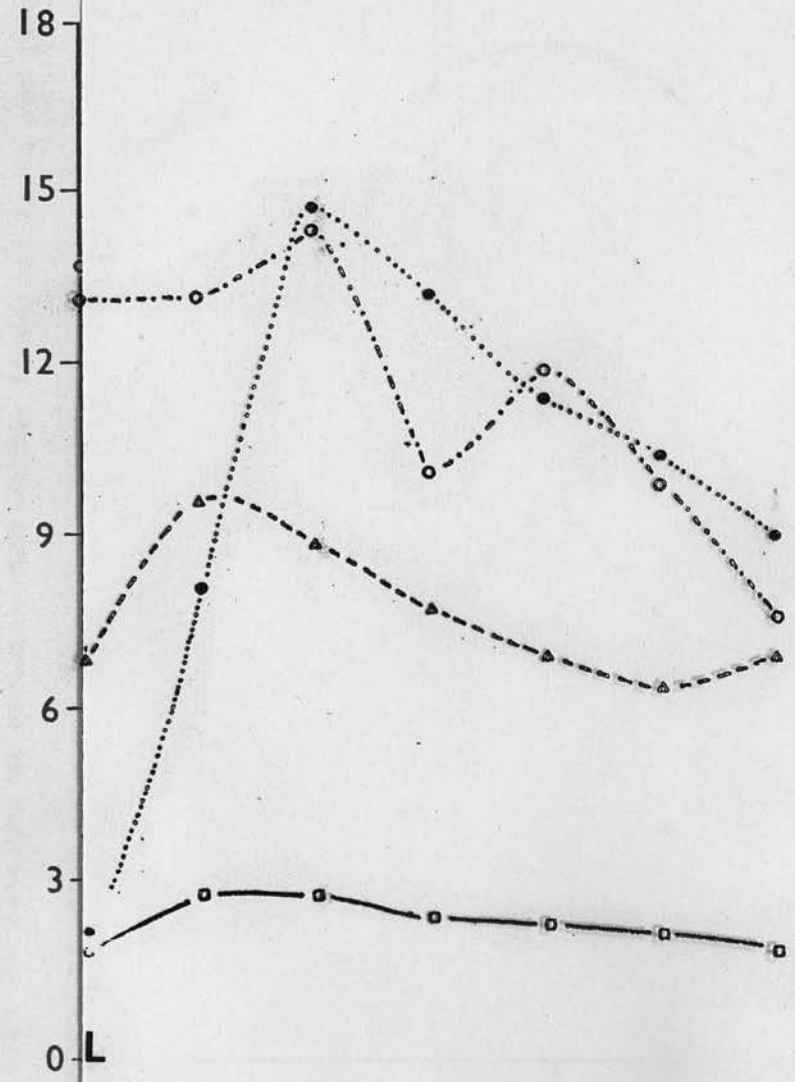


Fig. 31

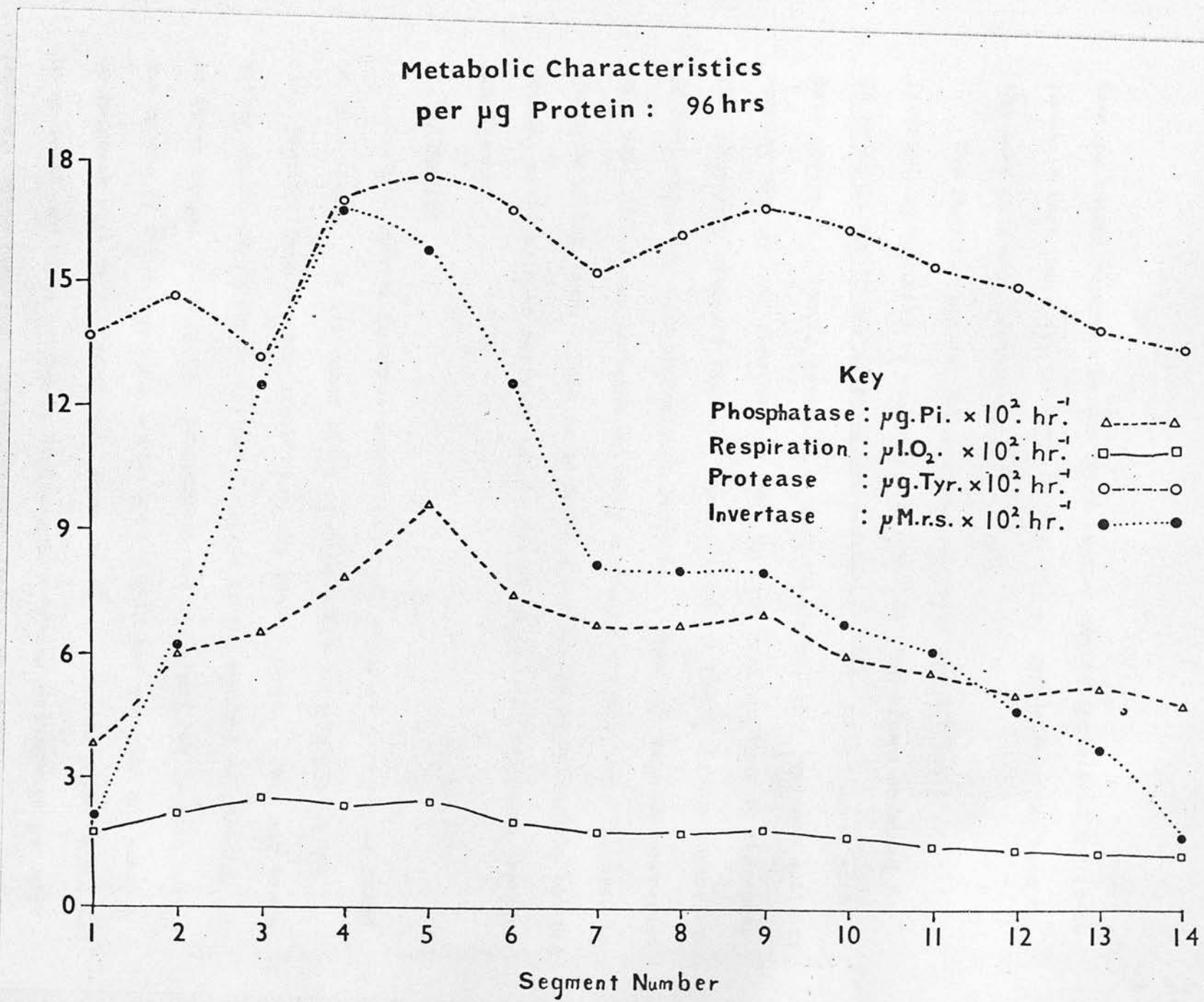

Metabolic Characteristics
per µg Protein : 96 hrs
18
15
12
9
6
3
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Segment Number
Key
Phosphatase : µg.Pi. × 10². hr.⁻¹
Respiration : µl.O₂. × 10². hr.⁻¹
Protease : µg.Tyr. × 10². hr.⁻¹
Invertase : µM.r.s. × 10². hr.⁻¹

Fig. 31

The low values recorded in the third segment may be fortuitous and it is probable that there is, in fact, no significant change from the base to the apex on a unit protein basis.

The results recorded for protease activity are difficult to interpret since there is reason to believe that the values obtained for S4 to S7 in the 72 hr. leaf are not reliable. They do not agree with data presented later in Series 2. With the 96 hr. leaf protease activity remains more or less constant per unit protein from the base to the apex. This constancy suggests that the protein added to the different organelles of the system may have proteolytic activity. When the data are expressed on a unit cell basis protease activity increases steadily from the base to the apex of the leaf. This is no doubt a reflection of increasing protein content with protease activity in all fractions of the increasing protein complement.

Discussion

Certain general features suggested by the array of data may be noted at this stage. In all cases there is an increase in activity as the cell develops from the meristematic to the mature state. This may be due either wholly or partly to the net increase in the content of protein. In three cases: respiration, phosphatase and invertase, it is also partly due to the fact that specific activity is low in the meristem and tends to increase with cell extension. When the fully extended state is reached in at least one case, protease, there is no decrease in activity per unit protein. With two, respiration and phosphatase, the evidence suggests that the apparent decrease may be due to an increasing proportion of chloroplast protein.

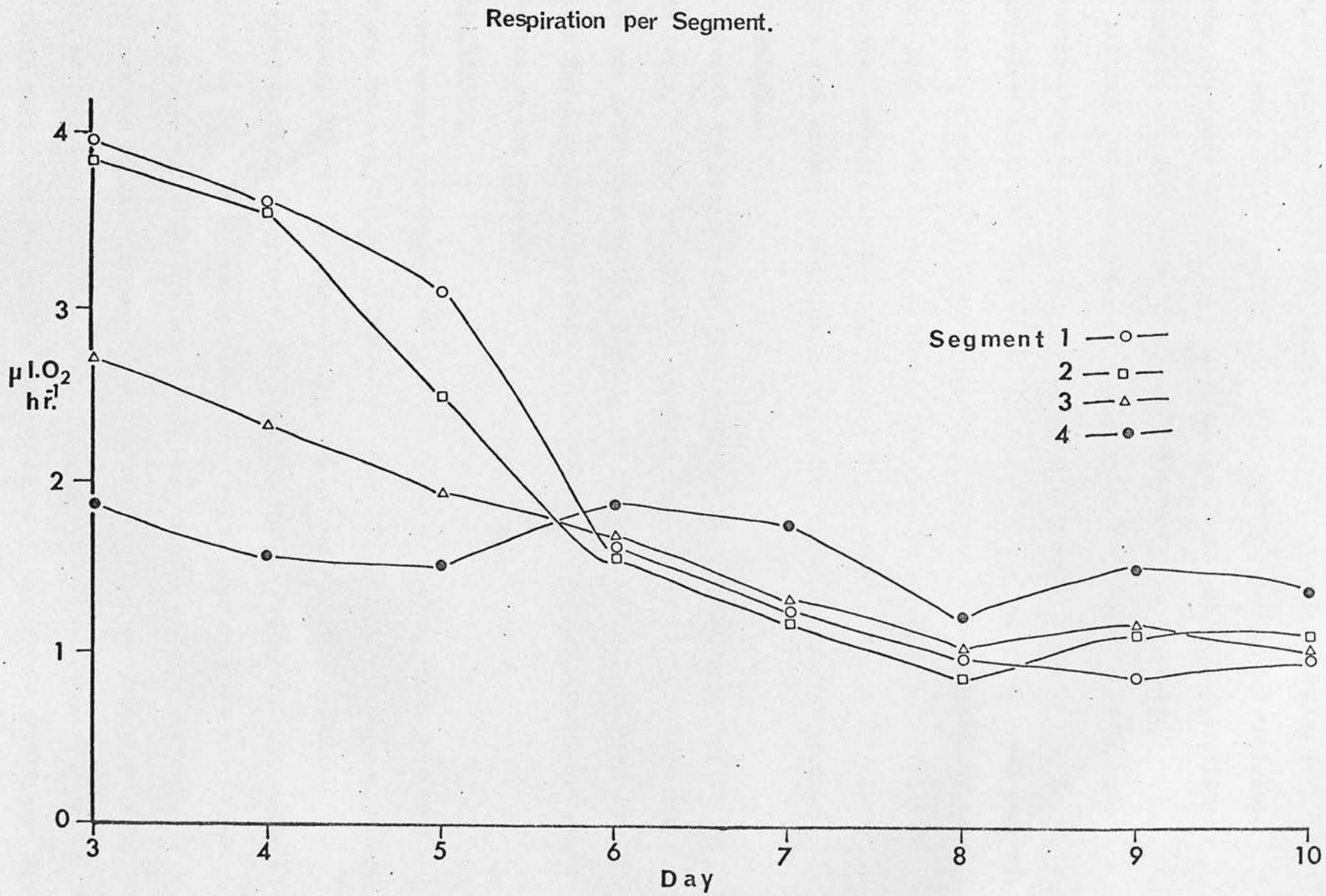

Respiration per Segment.
4
3
2
1
0
μ l.O2 hr.-1
Segment 1
2
3
4
3
4
5
6
7
8
9
10
Day

Fig. 32

Thus, in the wheat leaf, the position may be that when extension is complete some catalytic activities remain constant and do not decrease. However, the case of invertase suggests that there may be a significant decrease in the activity of others after expansion is complete.

Although the coincidence is not so close with regard to metabolic characteristics as it was for quantitative characteristics, it is nevertheless clear that the activities recorded for the 72 hr. leaf compare, in general, closely with those found for the first seven segments of the 96 hr. leaf. Thus it may be stated that the development of the cells of successive segments of the leaf at both 72 and 96 hrs. follow a similar pattern with regard to both quantitative and metabolic characteristics.

Series 2

Results are presented of determinations on the same characteristics as in Series 1 with the addition of photosynthetic activity. Separate graphs are given showing the activity per segment, per cell and per unit protein for each characteristic. Tables of the original results are again presented in the Appendix.

Respiration

The change in respiration rate in the four segments taken from each leaf are shown in Fig.32. These data show that the highest initial values for respiration rate per segment are given by the two basal segments. The third occupies an intermediate position and the lowest initial value is given by S4. In S1 and S2 respiration falls sharply until day 6, more slowly between day 6 and day 8 and then remains more or less constant. The values for S3 fall steadily from the third day to the eighth and then remain more or less constant. With S4, there is little change between the third and tenth day; there are minor fluctuations which may not be significant and the final value is lower than the first.

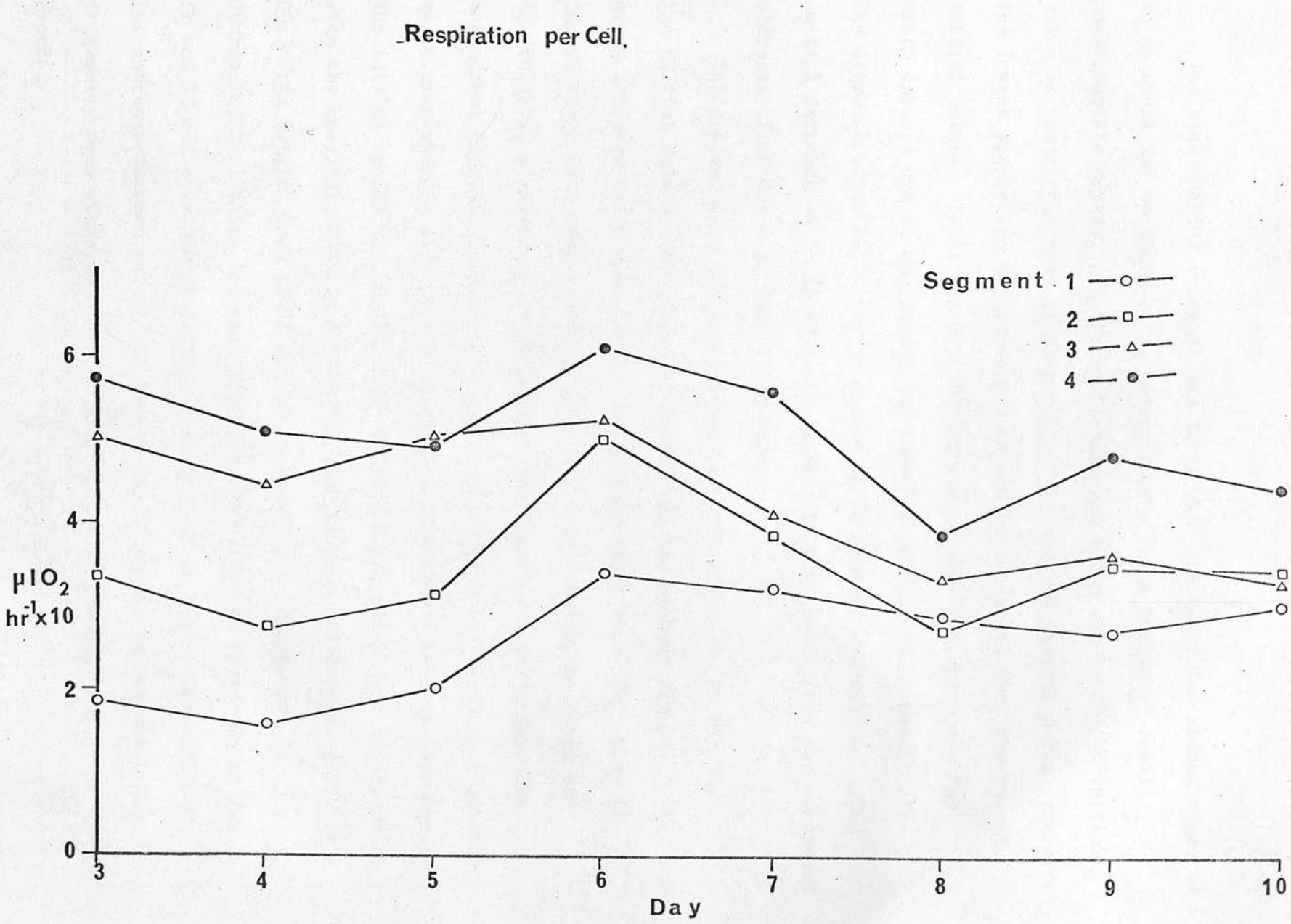

Respiration per Cell.
Segment 1
2
3
4
6
4
2
0
µlO2
hr-1x10
3
4
5
6
7
8
9
10
Day

Fig. 33

The high initial value on day three with the first and second segments is no doubt due to these being composed of a large number of small non-vacuolate cells. In S3 vacuolation has begun and hence the initial value is lower than that of S1 and S2. S4 has the largest cells, therefore, the lowest proportion of active protoplast material and also the lowest initial value. In S1 and S2, the decline between the third and the sixth days is due to the increasing number of highly vacuolated cells. The decrease after day 6 may be discussed in another connection. The initial decrease in S2 is no doubt associated with expansion, the continued decrease after day 6 is considered below.

The derived data for respiration per cell are given in Fig.33. Of the initial values, the lowest is for S1 and the highest for S4. S2 gives a higher value than S1 and S3 a higher value than S2. With S1 and S2 there is a drop in the respiration rate between the third and fourth days, a marked increase between the fourth and sixth days and a subsequent decrease to about the eighth day. With S3, there is a similar succession whereas with S4 the values probably remain more or less constant. The initial values are in the order of cell size, the lowest values being with the smallest cells in S1 and the highest with the largest cells in S4. The initial drop in S1 and S2 is probably a consequence of decreasing cell size, the rise from the fourth to the sixth day in S1, S2 and S3 reflects the progressive vacuolation in these segments. The decrease between days 6 and 8 with S1, S2 and S3 is one which has frequently been observed when the mature size of the cell has been reached.

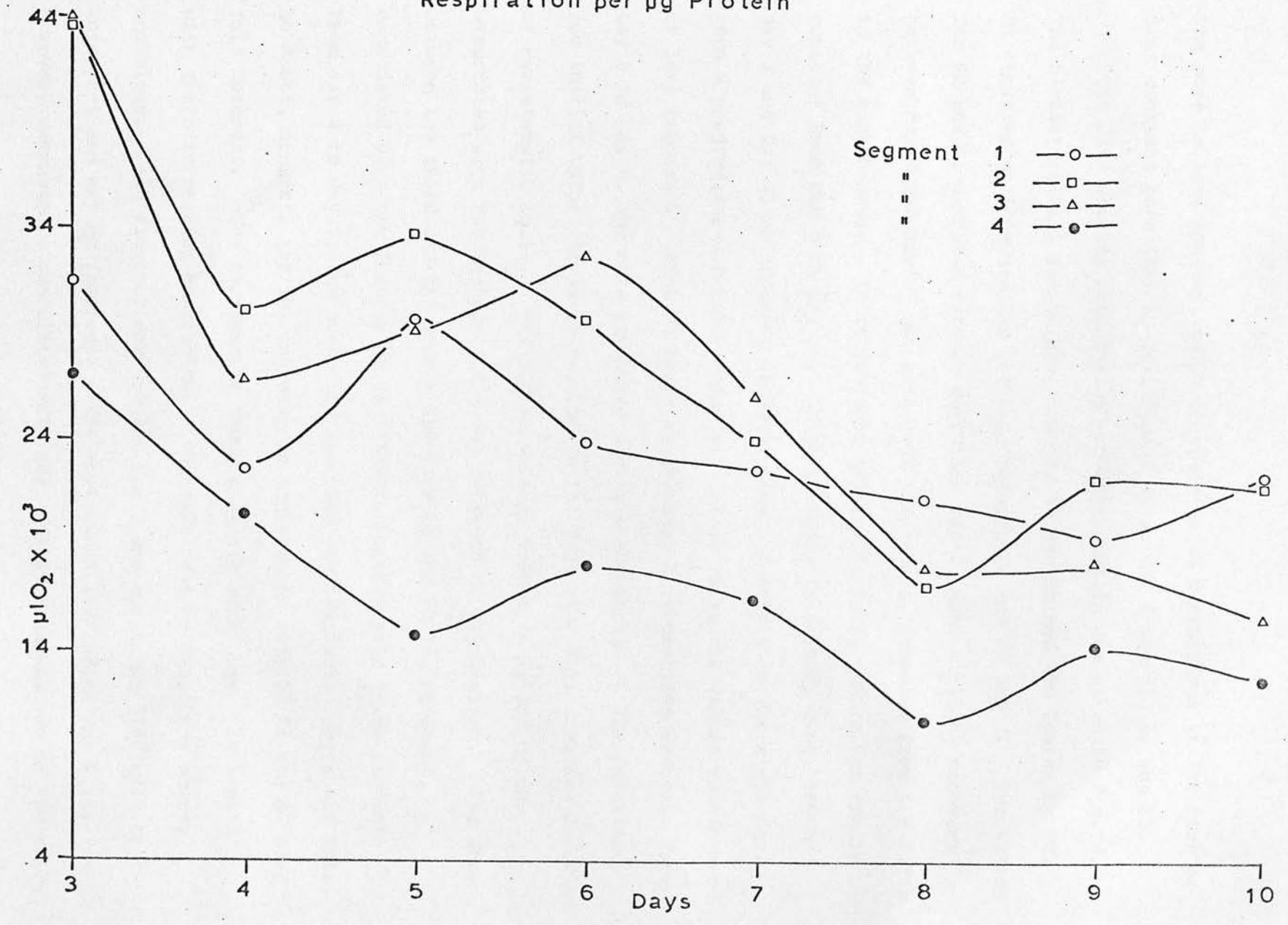
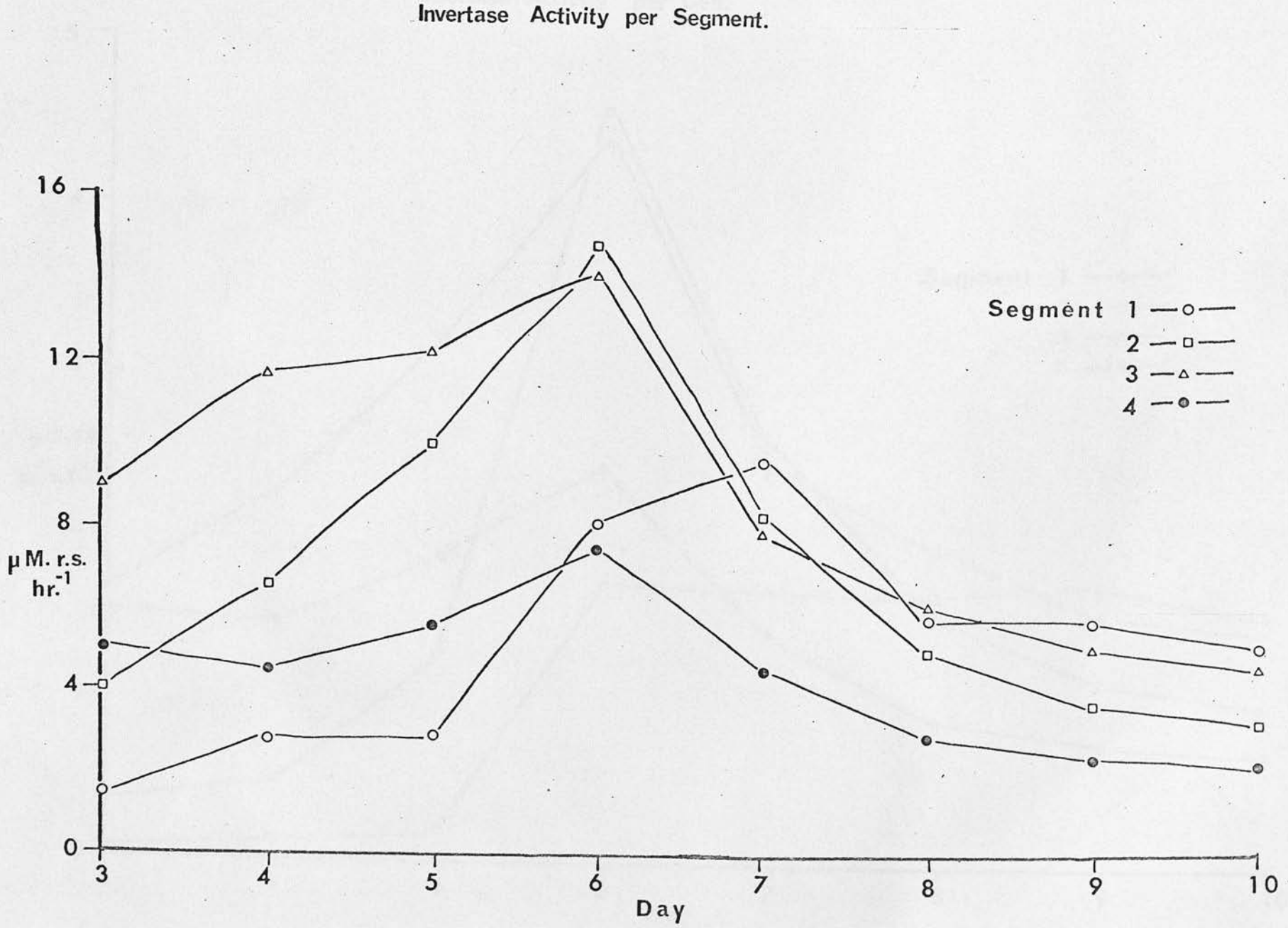

Respiration per µg Protein
Segment 1
" 2
" 3
" 4
44
34
24
14
4
µlO2 X 10³
3
4
5
6
7
8
9
10
Days

Fig. 34

The more or less constant value of cells in S4 corresponds to the more or less constant rate that is established after day 8 for S1, S2 and S3.

The data showing respiration per unit protein are given in Fig.34. The highest initial values are given by S2 and S3 and the lowest by S4. S1 occupies an intermediate position between S4 and S2 and 3. The values for S2 and S1 decrease between day 3 and day 4, make a slight recovery between day 4 and day 5, but with both the values, decrease from the fifth to the eighth days. It is probable that, with both, the values remain constant from day 8 to day 10. With S3 there is a sharp drop between day 3 and day 4, an apparent recovery from the fourth to the sixth day, then a progressive decrease to day 8. After this, the values remain more or less constant. With S4 there is probably a progressive decrease from day 3 to day 8, the rate remaining constant thereafter. The relatively low initial value for S1 is related to the fact that this consists largely of meristematic cells. The higher initial values in S2 and S3 are associated with the beginning and the progress of vacuolation. The drop between the third and the fourth day with S1 and S2 is, no doubt, associated with the increasing meristematic activity in these segments. From day 4 to day 5, the cells are becoming progressively larger and this, no doubt, accounts for the increase in respiration rate in S1 and S2 over this interval. The decrease in these segments after day 5 is linked with their increasing maturation. The data from S3 are, in a sense, anomalous. The fluctuations between day 3 and day 6 are difficult to interpret and may be spurious. The continuous fall after day 6 is, however, undoubtedly associated with the continued maturation of the cells.

Fig. 35


Invertase Activity per Segment.
16
12
8
4
0
µM. r.s. hr.-1
3
4
5
6
7
8
9
10
Day
Segment 1
2
3
4

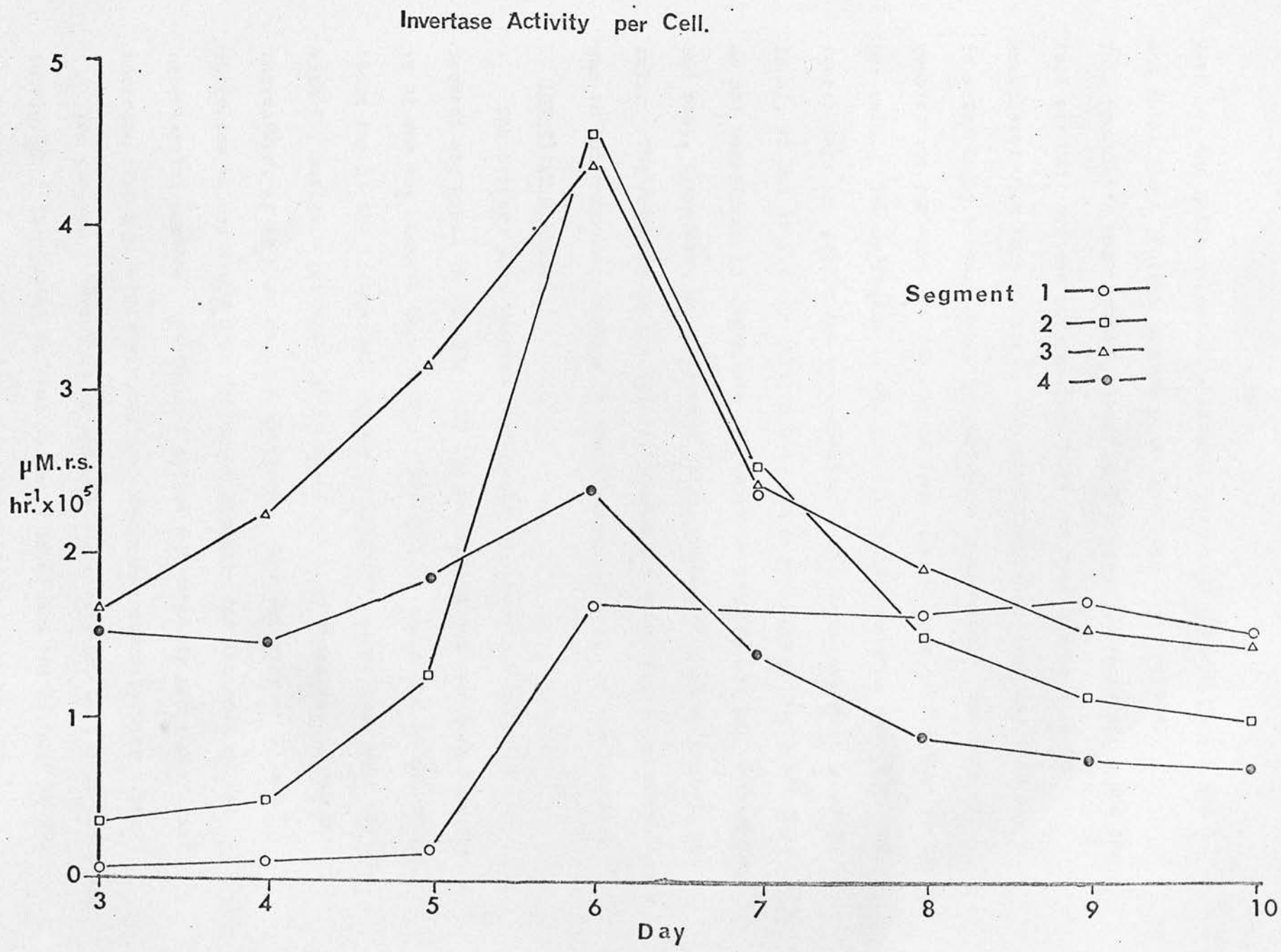

Invertase Activity per Cell.
5
4
3
2
1
0
μM.r.s.
hr.⁻¹x10⁵
Segment 1
2
3
4
3
4
5
6
7
8
9
10
Day

Fig. 36

With S4, the cells expand in a lateral direction between days 3 and 4 and chloroplast protein increases in them over this interval. This protein is most probably inert with respect to respiration and the fall per unit protein between the third and fourth days will be associated with this factor. The continuous fall from day 4 to day 8 is undoubtedly a consequence of continued maturation. The data of respiration per unit protein are of some significance in relation to those per cell. The decreases in the per cell values between the third and fourth days are partly due to decreases in the value per unit protein. In S1, S2 and S3 the per cell values, which increase to the sixth day, do not correspond to comparable increases in respiration per unit protein and must, therefore, be attributed to an increasing mass of protein per cell. The decrease in the values between day 6 and day 8 are undoubtedly due to corresponding changes in the specific activity of the protein.

Invertase activity

The primary data showing the changes in invertase activity per segment are given in Fig.35. Of the initial values the lowest is that in S1 and the highest that in S3. The initial value for S2 is between those for S1 and S3 and that for S4 is slightly higher than that for S2. With S1, activity increases up to day 7 and then decreases to day 8; thereafter, it is more or less constant. The activity of S2 and S3 increases to day 6 and then decreases steadily for the rest of the experimental period. S4 shows a slight but probably not significant increase from day 4 to day 6 and then decreases steadily with time.

The derived values showing invertase activity per unit cell are given in Fig.36. The lowest initial value is in S1 and the highest in S3. S2 is slightly greater than S1, and S4 slightly lower than S3.

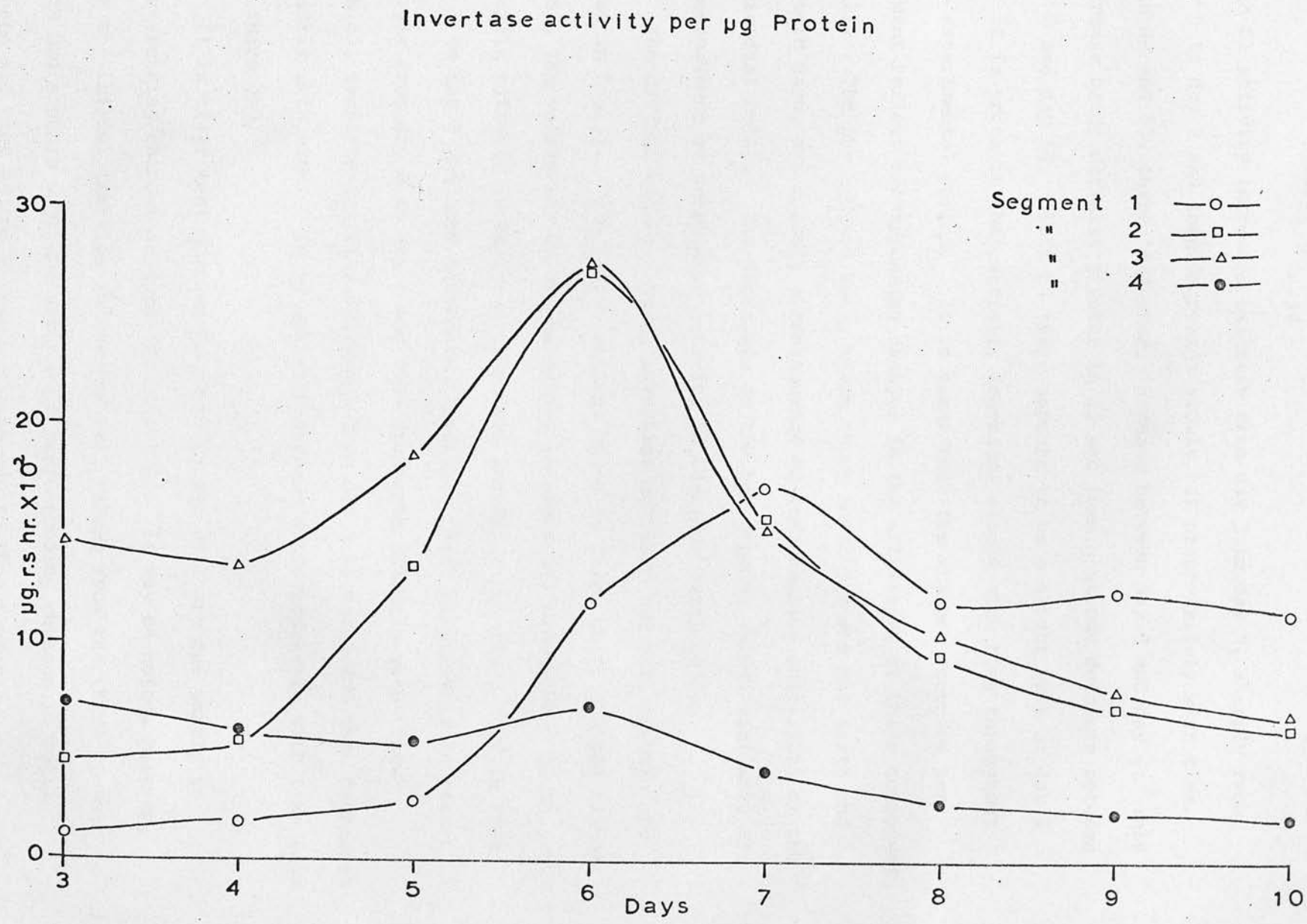

Invertase activity per µg Protein
Segment 1
" 2
" 3
" 4
µg.r.s hr. X10²
30
20
10
0
3
4
5
6
7
8
9
10
Days

Fig. 37

With S1 activity increases slightly from day 3 to day 5, sharply from day 5 to day 7 and then decreases slowly but progressively with time. With S2 and S3, there is a sharp increase between day 3 and day 6; this increase being initially greater in S3 and then a steady decrease between day 6 and day 10. With S4 there appears to be a slight peak at day 6 but it is probable that activity decreases slowly with time throughout the experimental period. It is clear that the changes recorded per segment reflect corresponding changes in the activities of their component cells. The per segment data, which shows increases for the sixth and seventh days, are clearly a consequence of the changing activities of the individual cells. The decreases in the per segment values similarly are a consequence of decreasing activity in the individual cells.

The derived values showing invertase activity per unit protein are given in Fig.37. The lowest initial value is given in S1 and the highest in S3, the values for S2 and S4 occupy intermediate positions. In S1 specific activity increases slowly over the first two days, sharply from day 5 to day 7 and then decreases steadily. With S2, activity increases sharply from day 4 to day 6 and then decreases steadily with time. With S3, specific activity increases from day 4 to day 6 and then decreases steadily with time. In S4 activity decreases progressively with time from the third day.

It is clear that changes in activity per cell are due partly to corresponding changes in specific activity. It may be noted, however, that the initial increase in the per cell values from the third to the sixth and seventh days are similar to corresponding changes in total protein and part of the increase may be due to this factor.

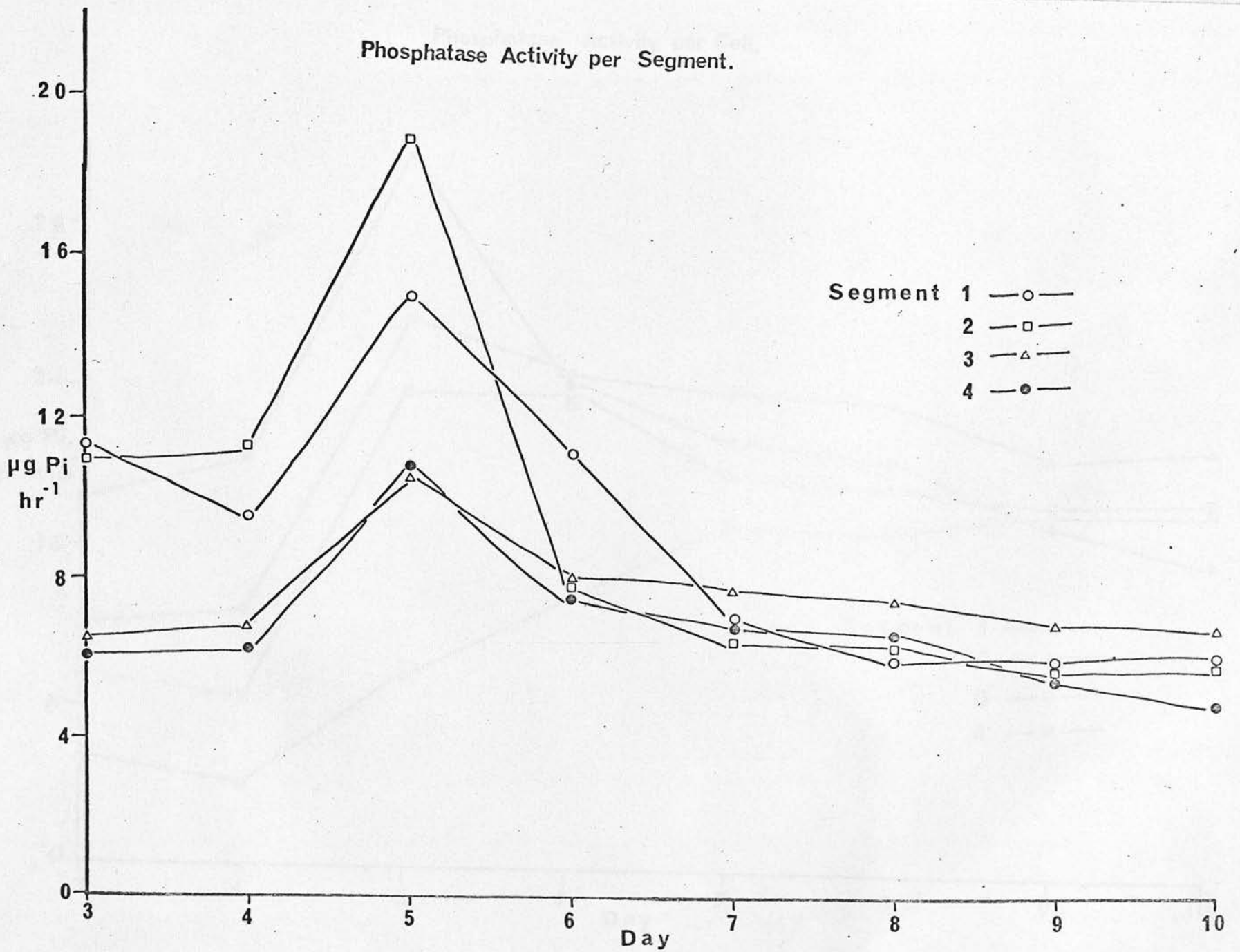

Phosphatase Activity per Segment.
20
16
12
8
4
0
µg Pi
hr-1
Segment 1
2
3
4
3
4
5
6
7
8
9
10
Day

Fig.38

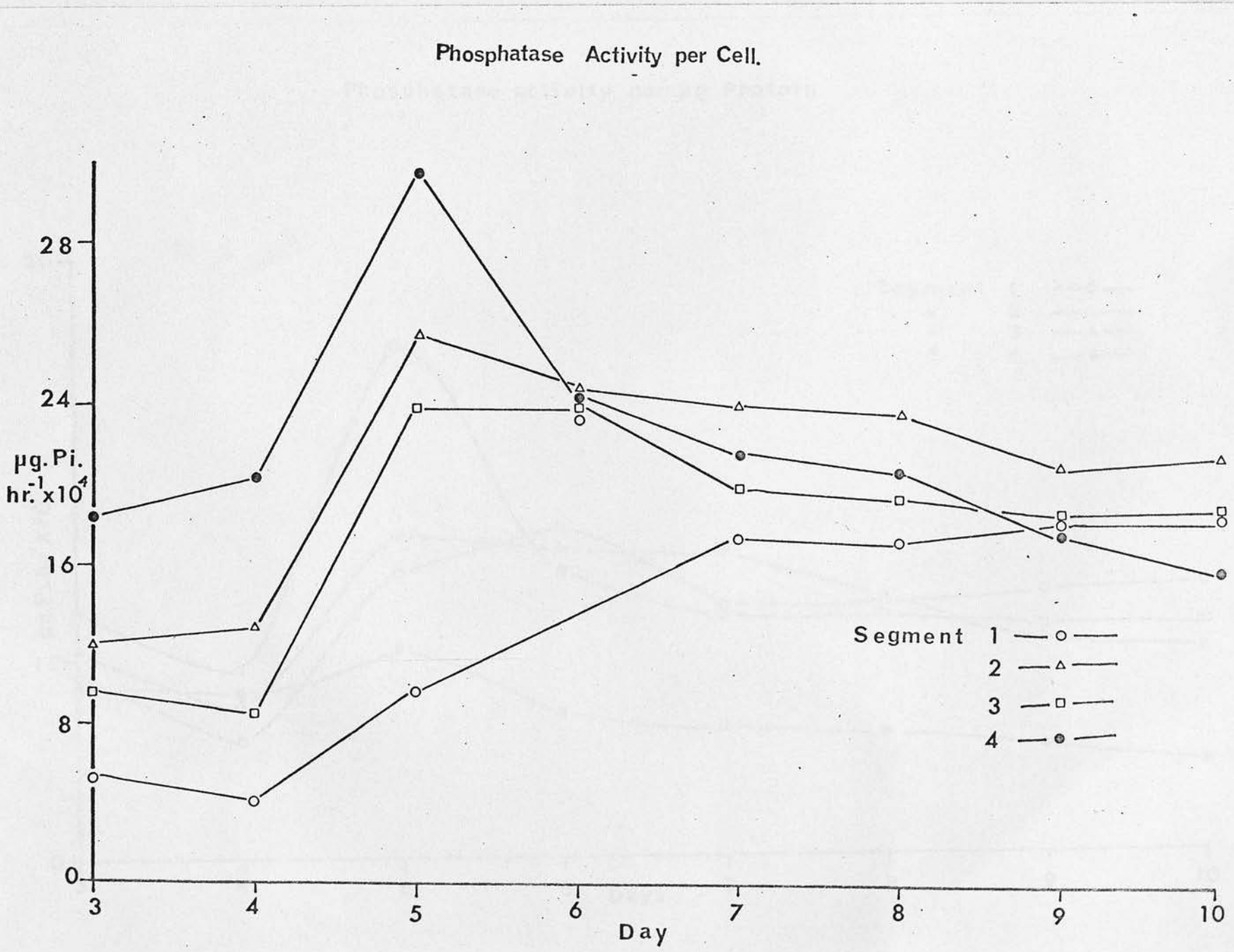

Phosphatase Activity per Cell.
28
24
16
8
0
µg. Pi.
hr.-1 x10-4
3
4
5
6
7
8
9
10
Day
Segment 1
2
3
4

Fig.39

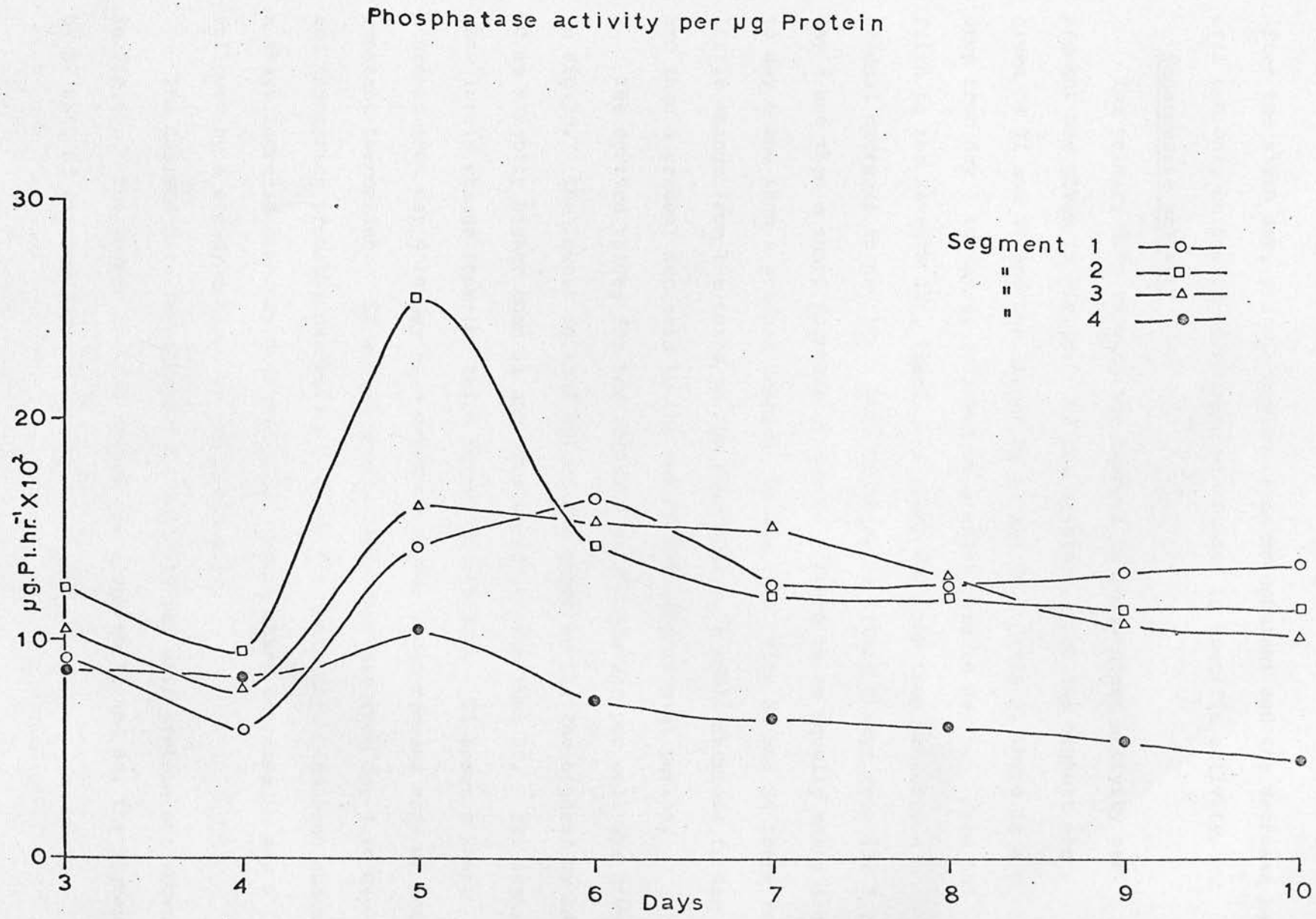

Phosphatase activity per µg Protein
Segment 1
" 2
" 3
" 4
µg.P1.hr.-1 X10-2
30
20
10
0
3
4
5
6
7
8
9
10
Days

Fig.40

After the sixth day, protein content remains constant and the decrease per cell can only be due to corresponding changes in specific activity.

Phosphatase activity

The primary data showing the changes in phosphatase activity per segment are given in Fig.38. Of the initial values the highest are given by S1 and S2 and the lowest by S3 and S4. With S1 there is a drop from day 3 to day 4, followed by a sharp rise to day 5. From the fifth to the seventh day, there is a sharp fall and then probably a gradual decrease to day 10. With S2 there is little change from day 3 to day 4 and then a sharp increase to day 5. There is an equally sharp decrease to day 6 and then a gradual decrease to day 10. With S3 and S4 there is little change from the third to the fourth days, a small increase to day 5 and then a gradual decrease to the end of the experimental period.

The derived values for the activity of phosphatase per cell are given in Fig.39. The lowest initial values are given by S1, the highest by S4; S2 is slightly higher than S1 and S3 slightly higher than S2. All segments show little change from the third to the fourth day. S1 shows a sharp increase from day 4 to day 6, a decrease to day 7 and remains more or less constant thereafter. S2 and S3 show a sharp increase from day 4 to day 5 and thereafter probably decrease slowly to the tenth day. S4 also shows a sharp increase from day 4 to day 5 but then a sharp decrease to day 6 followed by a steady decline to the tenth day.

The derived data for phosphatase activity per unit protein are shown in Fig.40. The lowest initial values are given by S1 and S4, the highest by S2 with S3 intermediate.

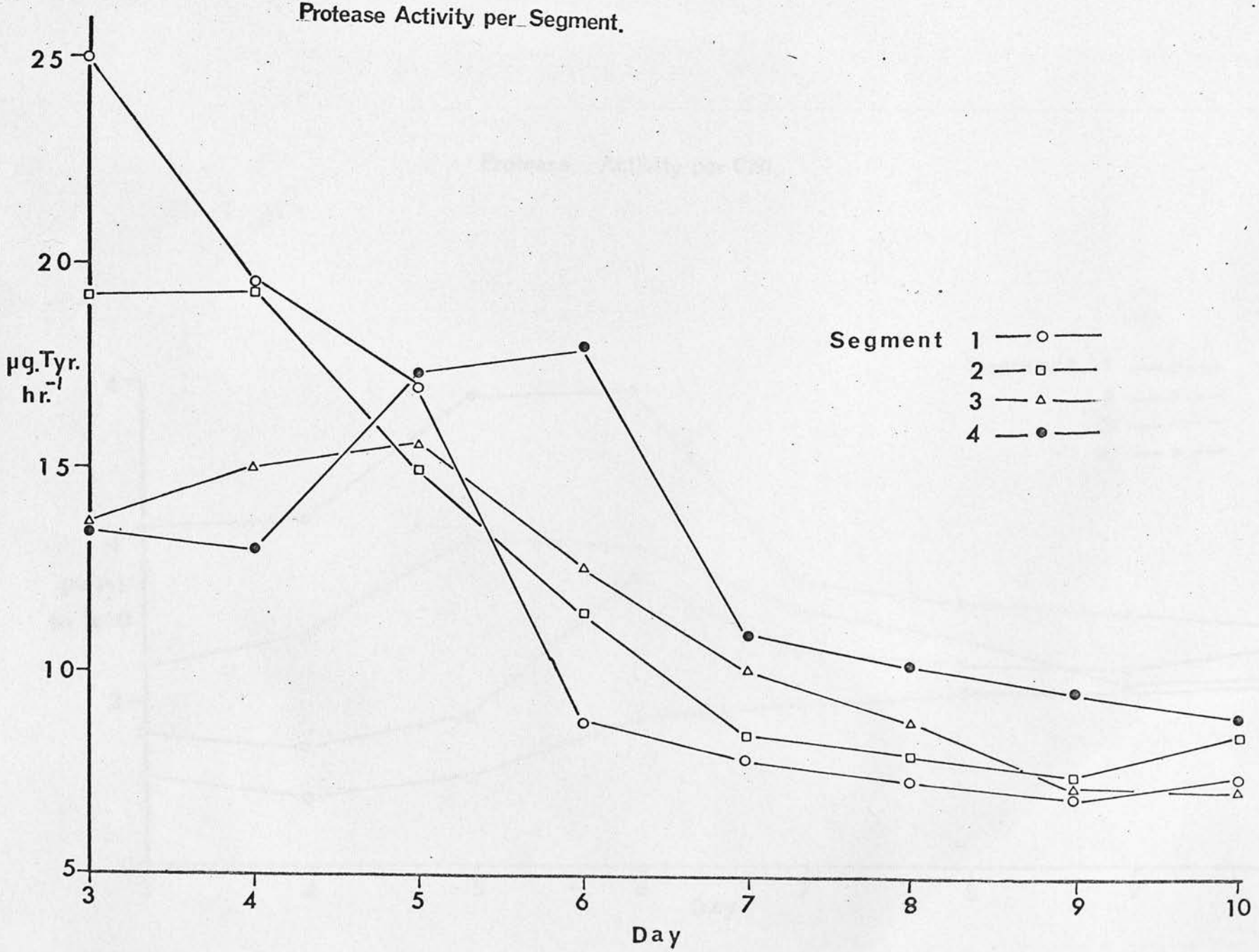

Protease Activity per Segment.
25
20
15
10
5
µg.Tyr. hr.⁻¹
Segment 1
2
3
4
3
4
5
6
7
8
9
10
Day

Fig.41

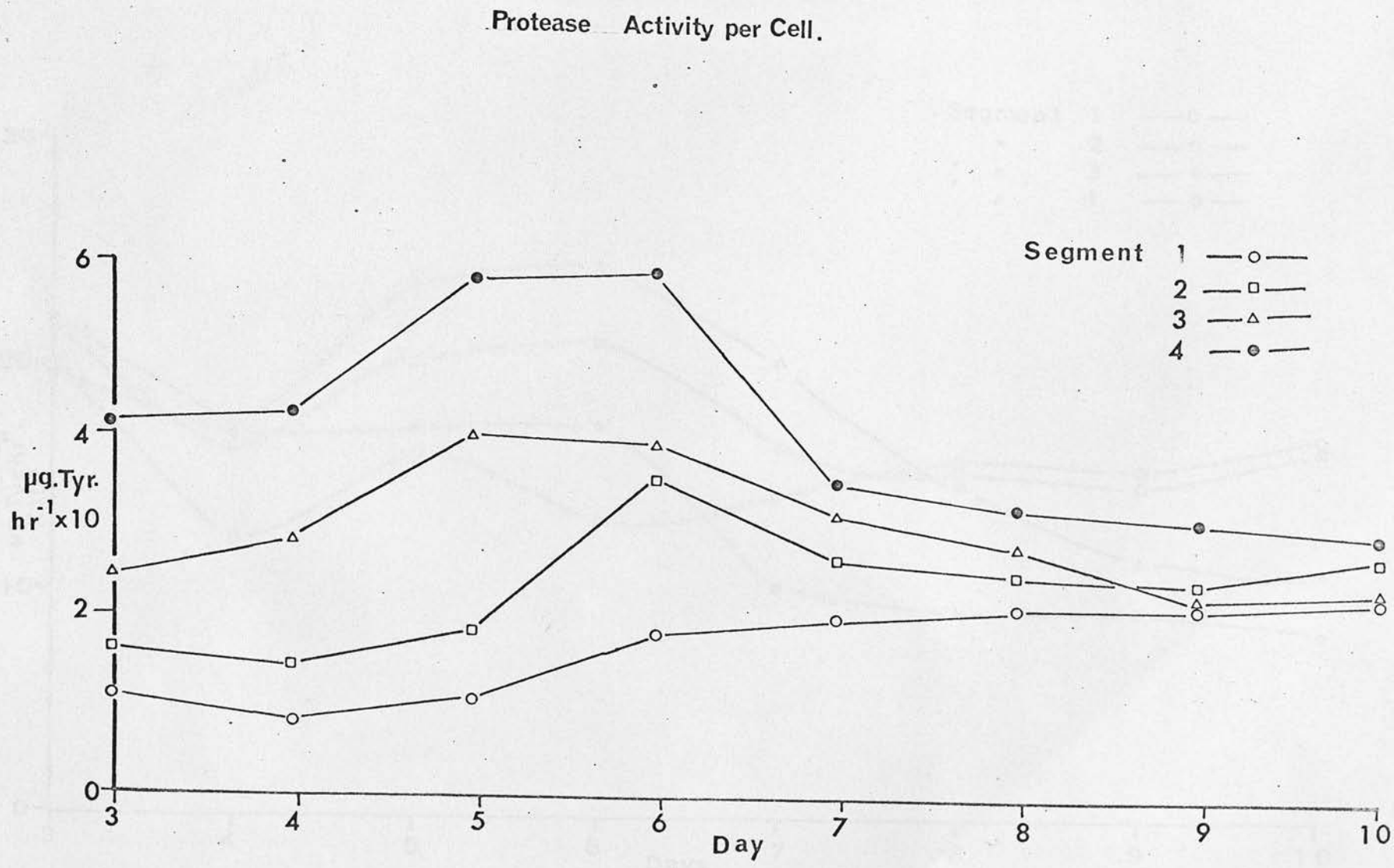

Protease Activity per Cell.
Segment 1
2
3
4
6
4
2
0
µg.Tyr.
hr⁻¹x10
3
4
5
6
7
8
9
10
Day

Fig.42

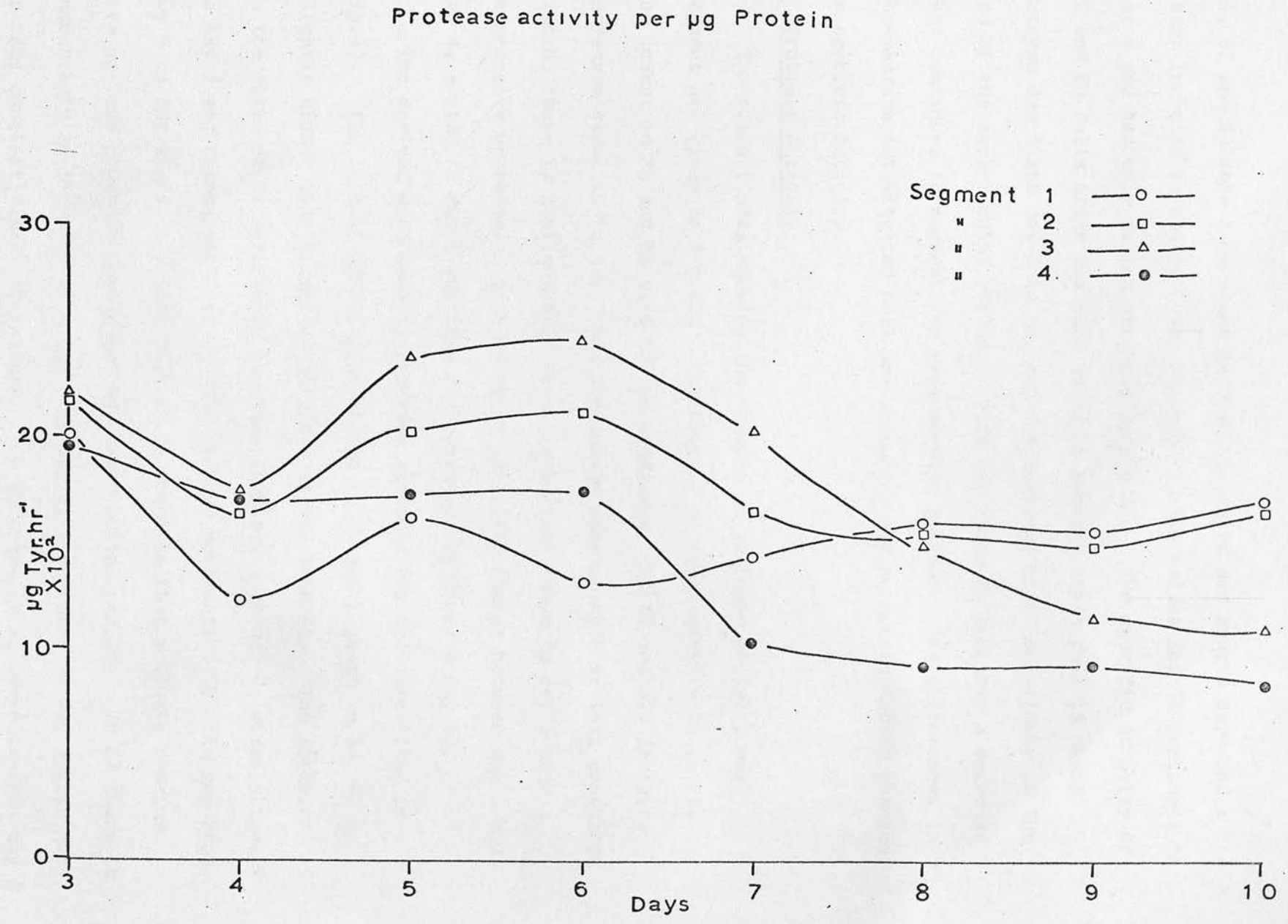

Protease activity per µg Protein
Segment 1
" 2
" 3
" 4
30
20
10
0
µg Tyr.hr⁻¹ X10²
3
4
5
6
7
8
9
10
Days

Fig.43

S1, S2 and S3 show a decrease between the third and fourth days and a sharp increase to day 5. The increase in the values for S1 continues to day 6 and has only fallen slightly by day 10. The specific activity of S2 and S3 falls after the peak value is reached, this fall is sharp between day 5 and day 6 in S2, and the decrease continues slowly to the end of the experimental period. With S4, there is probably a decrease that continues throughout the experimental period. The differences in phosphatase activity per cell are primarily due to corresponding changes in specific activity.

Protease activity

The primary data showing the changes in protease activity per segment are given in Fig.41. The highest initial activity is in S1, the lowest in S3 and S4 with S2 intermediate. In S1 and S2, activity decreases steadily to day 7 and thereafter remains more or less constant. With S3 there is a slight rise from the initial value to day 5 then a progressive decrease. With S4 there is little change between day 3 and day 4, a rise to day 6 and then a progressive decline to day 10.

The derived data showing protease activity per cell are given in Fig.42. The initial activity is lowest in S1 and highest in S4, S2 is slightly higher than S1 and S3 slightly higher than S2. The activity in the cells of S1 apparently declines from day 3 to day 4, rises slightly to day 6 and thereafter remains more or less constant. The changes from day 3 to day 6 are so slight that it is possible that activity remains more or less constant throughout the experimental period. In S2 there is undoubtedly an increase from day 5 to day 6, a decrease to day 7 with more or less constant values thereafter. In S3 there is an increase from day 3 to day 5 followed by a decrease to day 10. In S4 there is an increase to day 6 with a progressive decline thereafter.

Photosynthetic Activity per Cell.

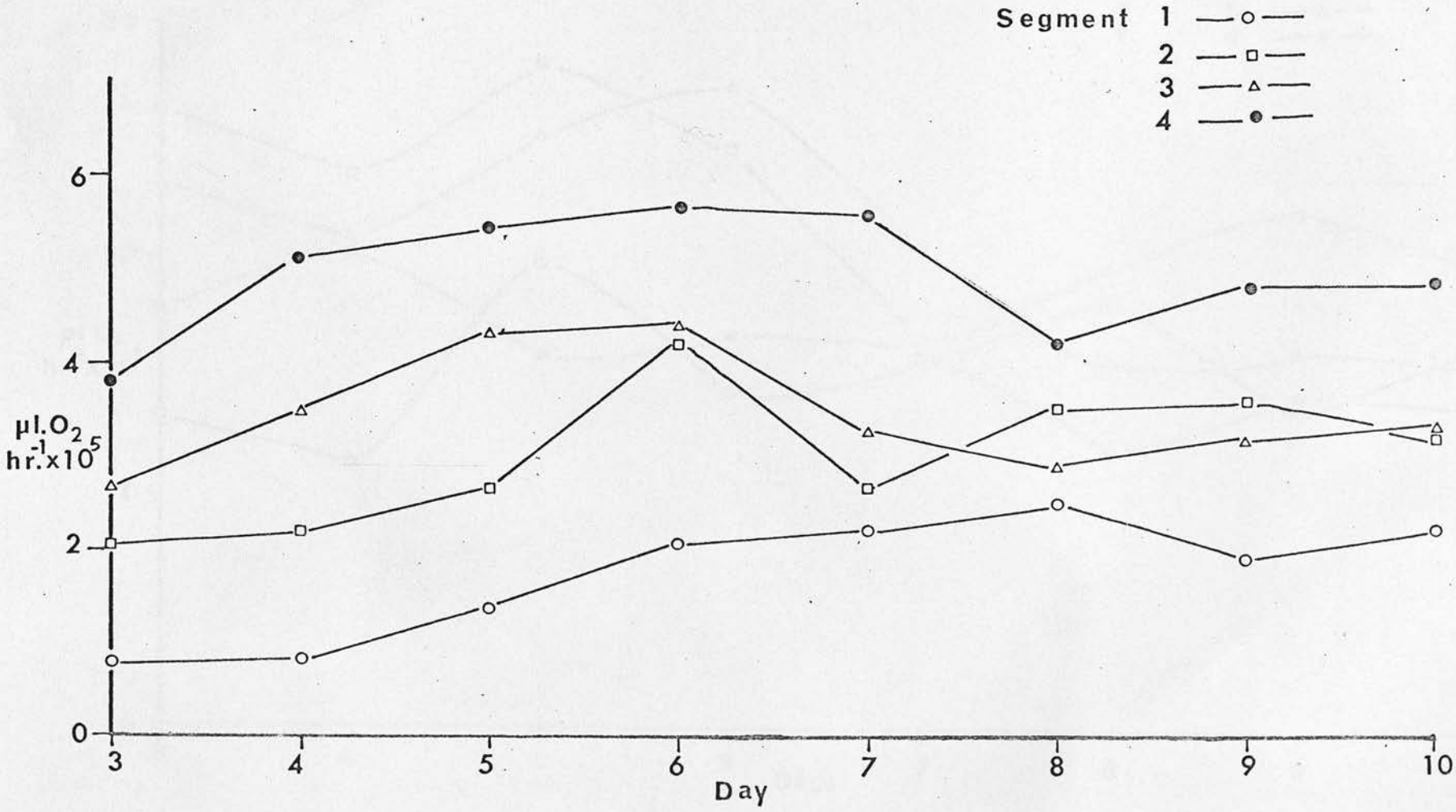


Fig.44

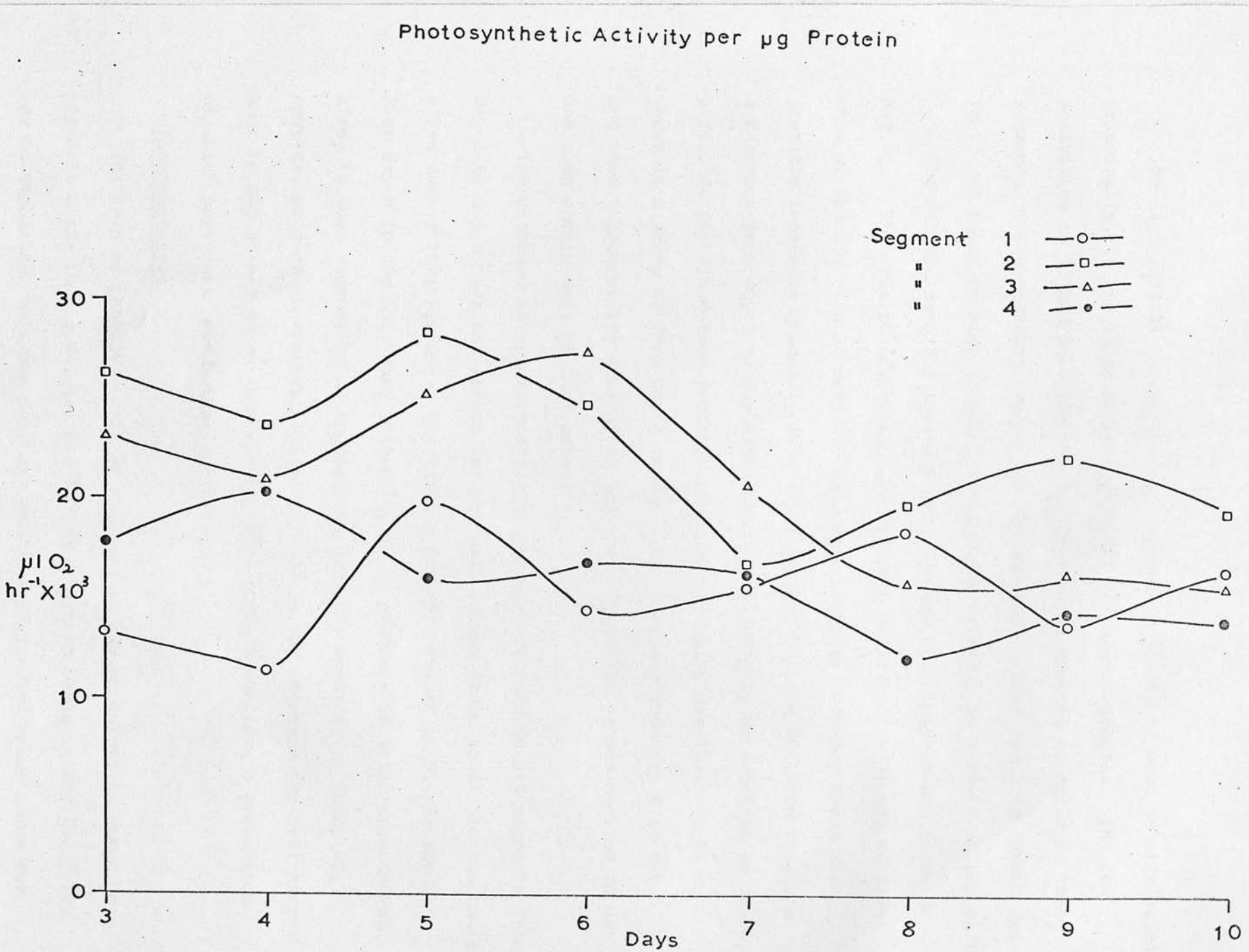

Photosynthetic Activity per µg Protein
Segment 1
" 2
" 3
" 4
µl O2 hr-1 X10³
30
20
10
0
3
4
5
6
7
8
9
10
Days

Fig.45

The high initial activities per segment in S1 and S2 must be attributed, principally, to the large numbers of cells in these segments. The low activities in S3 and S4 being due to the smaller numbers of cells in these segments. The increase in the per segment values from day 3 to days 5 and 6 in S3 and S4 is related to the corresponding increase in activities per cell.

The derived data for protease activity per unit protein are given in Fig.43. The initial activities are all very similar with S1 and S4 lower than S2 and S3. In S1 activity decreases from day 3 to day 4 and then probably increases gradually from day 4 to day 10. In S2 there is again a decrease from day 3 to day 4 but this is followed by an increase to day 6, a fall to day 7 and then probably constant values to day 10. In S3 there is a decrease from day 3 to day 4, an increase from day 4 to day 6 and then a progressive decline to day 10. In S4 the values decline to day 7 and then remain more or less constant.

The decrease in specific activity per unit protein in all segments from day 3 to day 4 does not affect the per cell values since total protein content rises during this period. The rise in per cell values in S1, S2 and S3 from day 4 to day 6 is due, primarily, to an increase in total protein but also, to some degree, to an increase in specific activity. There is, however, no rise in specific activity in S4 so the increase per cell here must be due solely to an increase in total protein content, a proportion of which possesses protease activity.

Photosynthesis

The rate of photosynthesis as measured by oxygen output is shown on a per cell basis in Fig.44, and on a per unit protein basis in Fig.45. It may be emphasised that the data are probably of limited value since the light intensity at the surface of the segments was low (500 ft. candles).

With such light limitation, changes with time are not likely to be significant. The data may, however, be considered in terms of the relative rates in the different segments since the order is likely to be the same at all light intensities. On a per cell basis, the highest rate is given throughout by S4; this rate being more or less constant from day 3 to day 10. S2 and S3 have rather similar curves with S3 initially higher. Both show a slight rise to day 6 followed by a fall to day 7 and then more or less constant values to day 10; the terminal values for these two segments being almost the same. S1 gives the lowest values of all throughout the experimental period; there appears to be a slight but progressive rise from day 4 to day 8 with constant values thereafter.

On a per unit protein basis, S4 has an intermediate initial value and fluctuates little from day 3 to day 10. S1 has the lowest initial value but shows a rise from day 4 to day 5 which is maintained with some fluctuation to day 10. S2 and S3 have the highest initial values, S2 rises further to a peak at day 5 and S3 at day 6. Both then decline with values becoming more or less constant after day 8. It is of interest to consider the data per unit protein with reference to those per cell. S4 shows a high value per cell but a fairly low value throughout per unit protein. It will be remembered that S4 had the highest protein content per cell, and it is, therefore, likely that S4 has the highest absolute amount of chloroplast protein per cell. S2 and S3 have both a greater total protein content than S1 and also a higher specific activity. These segments, therefore appear to have a greater amount of chloroplast protein than S1 and this is reflected in the per cell data.

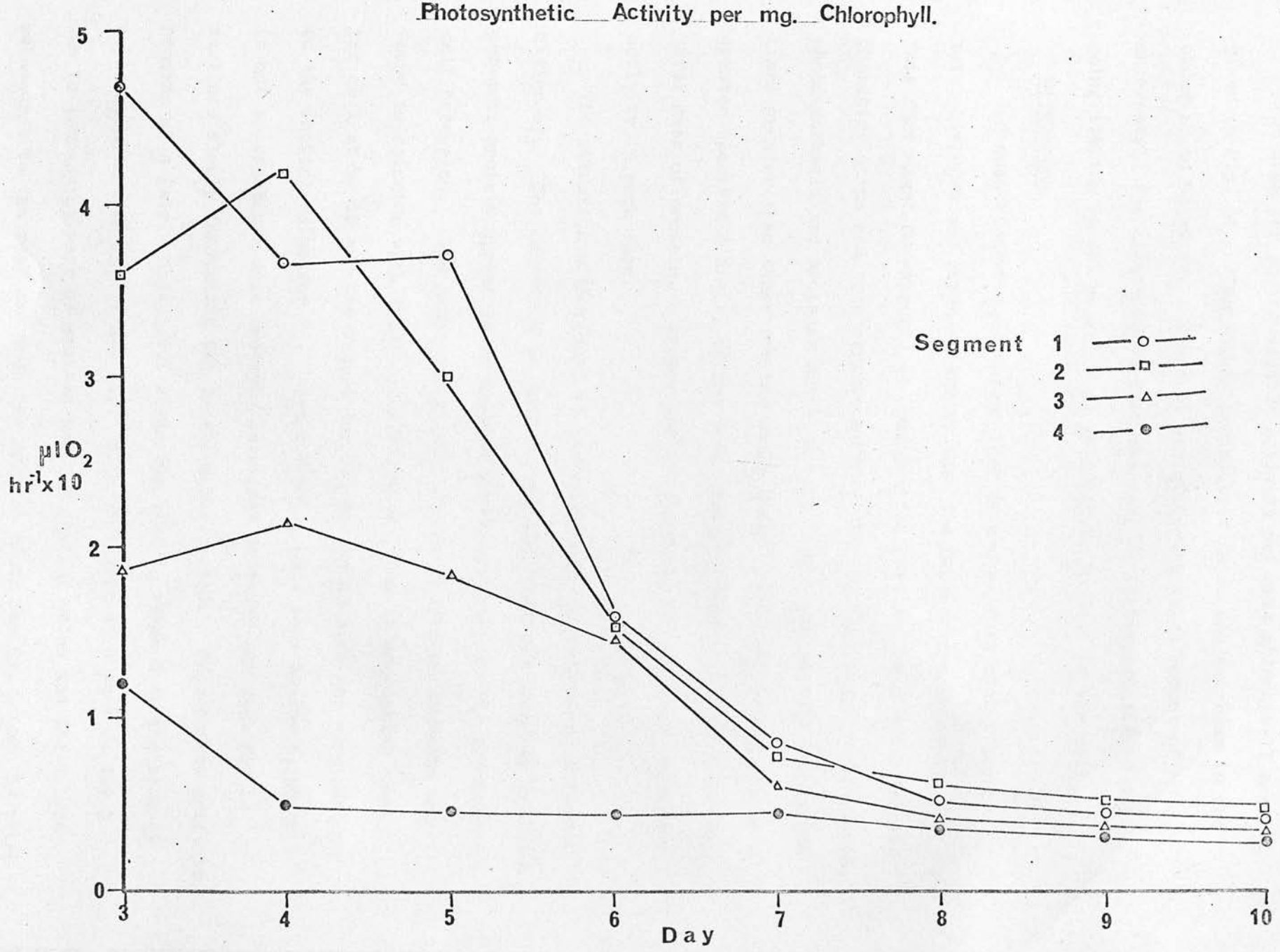

Photosynthetic Activity per mg. Chlorophyll.
Segment 1
2
3
4
$\mu l O_2$
$hr^{-1} x 10$
0
1
2
3
4
5
3
4
5
6
7
8
9
10
Day

Fig.46

The data for photosynthetic activity per unit chlorophyll are given in Fig. 46. The values are highest in S1 and decrease in the order S2 to S3 to S4. Since S1 contained the least amount of chlorophyll the data suggest that the rate of photosynthesis is not being limited by the quantity of chlorophyll present in the cell.

Discussion

The quantitative data showed that S4 had a considerably higher total nitrogen and protein content than the other three segments. This fact might be expected to dominate the per cell data for metabolic characteristics and, to a certain extent, this is the case. Respiration, photosynthesis and protease activity have values for S4 that are at all times greater than those for the other segments. This is not due to a greater specific activity of the total protein content but to a greater total mass of protein, a proportion of which has the relevant catalytic activity in each case.

The situation with regard to invertase and phosphatase is somewhat different. The increases in activity of these enzymes both per cell and per unit protein appear to be related almost entirely to the process of cell extension. The peaks of activity in the different segments are found to coincide with this. Invertase activity is decreasing slowly per cell at day 10 and the values for S1, S2 and S3 here are very similar to the initial value for S4. Apart from a small peak at day 6, which is not apparent per unit protein, invertase activity per cell for S4 declines slowly throughout the experimental period. Phosphatase activity remains at a fairly high level after the peak is reached at the time of rapid extension in S1, S2 and S3. With S4, there is a peak at day 5 due to increasing mass of protein but the initial value and the values subsequent to the peak are much the same as those for S1, S2 and S3 after the completion of expansion.

It appears, therefore, that in the fully extended cells the activities of invertase and phosphatase are not dependent on the total mass of protein.

With regard to the data of Series 1, the data of Series 2 confirm that all the metabolic characteristics measured increase per cell and per unit protein as the cells extend. However, with the exception of photosynthesis all metabolic activities appear to decline again once the maximum cell length is reached. In S4, the higher protein content may be responsible for maintaining these metabolic activities at a high rate after the attainment of maximum length.

DISCUSSION

Comparison of the two groups of data of Series 1 with those of Series 2, which it may be emphasised were performed with a different experimental arrangement, shows that they are clearly consistent with each other. The first series of observations are based on serial segments taken from the base of the leaf with leaves of two ages specifically 72 and 96 hrs. after germination. The experiments of Series 2 involved observations on three basal segments and one subterminal one taken at intervals of 24 hrs. between 3 and 10 days. Clearly a comparison may be made between the initial values in Series 2 and the values for the first three segments from the base of the 72 hr. and 96 hr. leaves. Further, the values for S5 at 72 hrs. and S12 at 96 hrs. may be compared with S4 at days 3 and 4 in Series 2. Thus the changes recorded in the first 24 hrs. in Series 2 may be compared with the changes recorded between 72 and 96 hrs. in Series 1.

The data of Fig.11 show that in the 72 hr. leaf fresh weight per cell, which may be taken as a measure of cell volume, increases steadily from S1 to S3 and the value for S5 is higher than that for S3. Fig.17 which shows the data for fresh weight per cell in Series 2 indicates that the lowest initial fresh weight on day 3 is in S1 and that the fresh weight increases with each successive segment of the series. Not only is the order the same in the two series of experiments but the absolute values are also similar. Thus, in the basal segment of the 72 hr. leaf in Series 1 (with the units being mgs. x 10^{-5}) the value is about 0.7 per cell and in S1 of Series 2, the initial value is about 0.8. In S2 of the 72 hr. leaf, the value is about 1.9 and in Series 2 about 1.8. The comparable figures for S3 are 4.5 and 3.8, and for the last segment they are 5.7 and 7.2. The values given second, are those for Series 2 and their greater size, undoubtedly, reflects the higher light intensity, temperature and longer daylength to which these leaves were exposed.

The three basal serial values for the 96 hr. leaf in Series 1 may be compared with the three basal segments of Series 2 on the fourth day. For fresh weight per cell, the comparable values (again in mgs. x 10^{-5}) are 0.6 and 0.9 for S1, 1.0 and 1.8 for S2 and 3.3 and 4.9 for S3. For S12 in the 96 hr. leaf of Series 1 and for S4 at 4 days in Series 2 the corresponding figures are 6.5 and 9.7. Clearly, on the fourth day the values for Series 2 are higher: this reflects, again, the more favourable growth regime to which the plants were exposed. After the fourth day, changes in Series 2 cannot be compared directly with data from Series 1. On the other hand, since the successive segments of Series 1 represent a time series, the trends shown in the later segments of the 96 hr. leaf may be compared qualitatively with the corresponding changes with time shown after the fourth day in S1, S2 and S3 of Series 2.

The dry weight changes along the course of the leaf in Series 1 are shown, per cell, in Fig.11. At 72 hrs., the dry weight values per cell increase from S1 to S3. The initial values in Series 2 also increase from S1 to S3. S5 of the 72 hr. leaf is considerably higher than S3 and this is shown again in the initial values of Series 2 where S4 is substantially greater than S3. In the 96 hr. leaf dry weight also increases progressively from S1 to S3 and S12 is considerably higher than S3. Comparable differences are shown in the data for the fourth day in Series 2 (Fig.19) and the absolute values for the two series of experiments are also comparable. From S1 to S3 in Series 1 at 72 hrs. the dry weight per cell (in mgs. x 10^{-5}) increases from 0.12 to 0.53 while in Series 2 the corresponding increase is from 0.13 to 0.42. The fifth segment of the 72 hr. leaf gives a value of 0.90, and S4 on day 3 of Series 2, a value of 1.04. For the 96 hr. leaf, the increase over the first three segments is from 0.12 to 0.51 and for day 4 in Series 2 it is from 0.12 to 0.47.

The twelfth segment in the 96 hr. leaf has a dry weight per cell of 1.18 and S4 at 4 days in Series 2 has a value of 1.04.

Total nitrogen per cell with the 72 hr. leaf (Fig.11) increases from S1 to S3, a similar change is shown by the initial values for Series 2 given in Fig.21. The fifth segment in the 72 hr. leaf has a higher value than the third which corresponds to the high initial value given by the fourth segment in Series 2. In the 96 hr. leaf, total nitrogen increases from S1 to S3 and S12 has a still higher value than S3. Corresponding differences are shown by the 4 day values for Series 2.

The change in the absolute values per cell for the first three segments of the 72 hr. leaf are from 1.45 to 3.58 (all values being in mgs. x 10^{-7}) and S5 gives a value of 4.51. The corresponding data for Series 2 are 1.18 to 2.38 for the first three segments and 4.25 for S4. The 96 hr. leaf shows a change from 1.5 to 4.03 between S1 and S3 and S12 gives a value of 6.63. The corresponding data for the fourth day in Series 2 are from 1.4 to 3.3 for the first three segments and 5.06 for S4.

The data of Fig.11 for the 72 hr. leaf show small but increasing values for chlorophyll content per cell in S1, S2 and S3. The values are also small in the three basal segments at day 3 in Series 2. The value for the fifth segment in the 72 hr. leaf is greater than that for S3, this is again reflected in the initial value for S4 at 3 days in Series 2 (Fig.28). The changes in the first three segments in the 96 hr. leaf is only slight and this is also shown by the fourth day values of Series 2. S12 has a considerably higher value than S3 in Series 1 and such an increase is also shown very markedly in the value for S4 for the fourth day in Series 2.

The absolute values for chlorophyll content per cell for the 72 hr. leaf show a change between the first and third segments from 0.07 to 0.99 (all values being in mgs. x 10^{-8}) the fifth segment gives a value of 1.56. The corresponding values for Series 2 are from 0.16 to 1.48 with S4 giving a value of 3.5. In the 96 hr. leaf the change from the first to the third segment is from 0.11 to 1.08 and the value for S12 is 5.8. The corresponding value for Series 2 is 0.22 to 1.65 and S4 gives a value of 15.41. Although the order is the same for the two series, clearly the absolute values differ considerably. This difference is again undoubtedly due to the fact that the plants of Series 2 were exposed to a higher light intensity and a longer daylength than those of Series 1.

Nucleic acid determinations were not made in Series 1 but from the data of Figs. 24 and 26, taken from Series 2, some indications may be gathered as to the position at 72 and 96 hrs. The initial values for RNA per cell (Fig.24) are lowest in the basal segment followed by S2 and S3, with S4 having the highest value. This suggests that in the 72 hr. leaf in Series 1, it is likely that the RNA per cell will increase from the first to the third segment and still further to S5. In the 96 hr. leaf there is probably a sharp increase in RNA per cell from S1 to S3 and thereafter it may remain more or less constant. Certainly in Series 2, the data for the fourth day indicate that RNA per cell is lowest in S1, increases sharply to S3 with S4 similar to S3. Determinations for DNA were made only on a limited scale in Series 2. The photometric measurements made on the first and fourth segments, however, suggest that there is little change in DNA content per nucleus either along the length of the leaf or with time. All the mean values recorded were approximately the same except for the basal segment during meristematic activity.

The high values recorded for this segment were undoubtedly due to measurements having been made on 2C, replicating and 4C nuclei whereas all the other values obtained are likely to have been derived from 2C nuclei only.

As with the basic quantitative data, the values for metabolic activity on a unit cell basis are again consistent for the two experimental series. Respiration in the 72 hr. leaf (Fig.30) increases from the basal to the third segment and there may be a slight increase from the third to the fifth. The initial values for Series 2 (Fig.33) are lowest at the base of the leaf with activity increasing from S1 to S2 to S3, from S3 to S4 and there may be a slight increase. The 96 hr. leaf in Series 1 shows activity increasing from S1 to S3 with the rate in S12 similar to that in S3. On the fourth day in Series 2, activity also increases from the first to the third segment with S4 slightly higher than S3.

Invertase activity in the 72 hr. leaf of Series 1 (Fig.30) increases sharply from S1 to S3 and thereafter decreases. A similar situation is found in the corresponding initial values for Series 2 (Fig.37), there is a steep rise again from S1 to S3 with activity per cell slightly lower in S4 than in S3. The 96 hr. leaf in Series 1 shows activity increasing from S1 to S3 but S12 is considerably lower than S3, again a similar situation is shown by the day 4 values for Series 2 - activity increasing from S1 to S3 with S4 rather lower than S3.

Phosphatase activity in the 72 hr. leaf of Series 1 (Fig.30) increases sharply per unit cell from S1 to S3 the value for S5 being slightly lower than that for S3.

Phosphatase activity in the 72 hr. leaf of Series 1 (Fig.30) increases sharply per unit cell from S1 to S3 the value for S5 being slightly lower than that for S3. The initial values for Series 2 (Fig.39) show the same increase from S1 to S3 but there is a further increase to S4. In the 96 hr. leaf, activity increases markedly from the first to the third segment and the value for S12 is greater than that for S3. The 4 day values for Series 2 show a similar situation with a sharp increase from the first to the third segment and a further sharp increase to S4.

Protease activity in the 72 hr. leaf (Fig.30) increases per cell from the first to the third segment and thereafter decreases, the value for S5 being lower than that for S3. The initial values for the second series (Fig.42) show an increase per cell from S1 to S3 but in this case there is also a marked increase from S3 to S4. It may be noted that, as indicated earlier, the protease values for the later part of the 72 hr. leaf may be unreliable. In the 96 hr. leaf of Series 1 protease activity per cell increases from S1 to S3 and then continues to increase the value for S12 being considerably higher than that for the third. A similar situation is shown by the comparable data for Series 2; on the fourth day, protease activity increases from the first to the third segment and then shows a further increase to S4.

Photosynthetic activities were not measured in Series 1 but the position may be inferred from the data of activity per cell in Series 2 (Fig.44). The initial values increase from S1 to S3 with a further increase to S4. This suggests that in the 72 hr. leaf activity increases from the base to at least S5. On the fourth day in Series 2, activity increases from S1 to S3 and the value for S4 is again higher than that for S3.

This suggests that for the 96 hr. leaf it is probable that photosynthetic activity per cell increases with distance along the leaf at least as far as S12.

The interpretation of the data of Series 1 and 2 requires some reference to the pattern of growth of the leaf from germination to the tenth day of seedling growth. At the time of germination, the first leaf is present as a primordium in the embryo, and by the end of the first 24 hrs. growth after germination the first leaf has developed from the primordium to a length of about 5 mm. It has grown to this length almost entirely by the proliferation of cells throughout the leaf and, at this stage, practically all the cells are in a meristematic state. They are small, non-vacuolate with large and prominent nuclei. At the end of 48 hrs. growth the leaf has reached a length of about 12 mm., much of this increase has been brought about by the onset of vacuolation in the apical cells, and this has spread downwards leaving a region of meristematic cells at the base. This meristem continues to divide and by so doing increases the number of cells in the leaf and thus the number available for vacuolation. An intermediate zone is evolved near the base of the meristem (probably between 48 and 72 hrs.) which is itself non-meristematic. This intermediate zone effectively divides the meristematic region into two. The lower group of meristematic cells become, in due course, the basal meristem of the leaf sheath; after about 72 hrs. this meristem produces cells upwards which, by vacuolation, induce the formation of a distinctive leaf sheath. The epidermal cells at the top of this intermediate zone differentiate to produce the ligule which demarcates the leaf sheath from the blade. This development is complete at 72 hrs. and at this stage a separate and distinctive lamina is available above the ligule.

At this stage also, there is a distinct intercalary meristem at the base of the leaf and above it cells which are in progressively advanced stages of differentiation and vacuolation. By 72 hrs., the lamina is 3.5 cm. long and between 48 and 72 hrs. meristematic activity proceeds with the continuous formation of cells which are progressively incorporated into the leaf. This process continues in the lamina until 96 hrs. when its length is about 7 cm. During the previous 24 hrs., active division has continued in the meristem and vacuolation has occurred in the cells which have been incorporated into the leaf. Further, some of the cells which started vacuolating before 72 hrs., complete vacuolation during this interval. In the 72 hr. leaf, the three terminal segments have been acquired from the apical 7 mm. of the 48 hr. leaf and by 72 hrs. these are fully extended. The basal four segments have been derived from the basal 5 mm. of the 48 hr. leaf. Of this basal group of four segments the first, next to the ligule, is wholly meristematic and the rest are in various stages of vacuolation. S5, S6 and S7 of the 72 hr. leaf do not grow further and become S12, S13 and S14 of the 96 hr. leaf. S2, S3 and S4 of the 72 hr. leaf become, through expansion, segments 6 to 11 of the 96 hr. leaf. In the 96 hr. leaf, the extreme basal segment is still meristematic but segments 2 to 5 have been formed in the previous 24 hrs. and are, at this stage, in the process of vacuolation. After 96 hrs. meristematic activity decreases sharply and although there may still be some divisions occurring at 120 hrs., they are few and division has ceased completely by 144 hrs. The continued increase in the length of the leaf after 96 hrs. is due primarily to the continued expansion of the cells present in S2 to S5. It is due also to the promotion of expansion in the cells of the previously active meristem.

At 6 days, when all meristematic activity has ceased, some expansion may still be continuing in the basal segment. However, after day 6 expansion in the basal segment does not greatly affect the length of the leaf, which is almost at its maximum at 6 days. The changes in the system between 6 and 10 days are virtually occurring in cells which have all reached their mature volume. It must be emphasised, however, that, as a result of the pattern of growth of the leaf, the cells at increasing distances from the base of the leaf must at all stages be regarded as being of increasing ages. Those at the base of the leaf are the youngest and those above them being progressively older.

At 72 hrs. the basal meristematic region contains about 210,000 cells, the next segment, S2, contains cells produced from this meristem and most of these will have just started vacuolating while some may still be dividing. The cells in S3 have also been produced from the meristem but at an earlier stage than those of S2 and have, therefore, extended further. The cells have also been produced from the meristem in the previous 24 hrs. at an earlier stage still and so are at an even more advanced stage of extension. Indeed it is probable that extension in this segment is more or less complete. In the 96 hr. leaf the basal segment is still meristematic but the second to the fifth segments have been derived from the meristem in the previous 24 hrs. S2 is the most recently formed and in it some divisions are still proceeding but vacuolation has begun. In the third segment, division has certainly ceased but here, the segment having been produced earlier, extension has proceeded further. Similarly in S4 since there is only limited increase in fresh weight after this it is probable that extension is more or less complete. In S5 to S11 extension is almost certainly complete and there is no further increase in length in the zone occupied by these segments.

The 3 and 4 day values in Series 2 confirm the corresponding values in Series 1. The significance of the two series of data is primarily in the indication they provide of the development of cells in various parts of the leaf. In S1 division is declining between the fourth and fifth days, this is accompanied by an increase in volume indicating that vacuolation begins as soon as division ceases in these cells. After the fifth day, division in the basal segment has more or less ceased, vacuolation continues, and, therefore, as shown in Fig.17 there is a sharp increase in fresh weight per cell between day 5 and day 6. Evidently all the cells of S1 do not begin vacuolation at the same time and as a result the average fresh weight per cell in S1 continues to increase between the sixth and eighth days. After the fourth day in S2 the effect of the declining rate of mitosis again becomes evident. There is a sharp increase in fresh weight per cell between days 4 and 5 and a further marked increase between days 5 and 6. In this segment all the cells have already started vacuolation on day 4 and this is, no doubt, the reason for the more or less constant fresh weight per cell after day 6.

After day 4, the cells in S3, which are being continuously derived from S1 and S2, and are in a high state of vacuolation continue to increase slightly in volume between the fourth and fifth days; and also with a new generation of cells between the fifth and sixth days. After day 6 there is no further change in volume in the cells of S3. With S4, which it may be recalled corresponds at 3 days to S5 of the 72 hr. leaf and at 4 days to S12 of the 96 hr. leaf in Series 1, extension appears to be complete at 4 days and no further change in fresh weight occurs. It is significant that the values between day 8 and day 10 are more or less the same in all segments.

The difference between the fresh weights in S1 and S2 and those in S4 between day 8 and day 10 is only a matter of about 10%. The position with regard to dry weight per cell is similar to that with fresh weight in the four segments of Series 2 (Fig.19). After day 4 in S1 dry weight increases slightly and sharply between the fifth and sixth days. After day 6, there is a slow increase in dry weight until day 8. With S2 and S3 there is a similar succession between the fourth and fifth days to that for fresh weight, i.e. with S2 a sharp increase between day 5 and day 6 and with S3 a slow increase between day 3 and day 6. With both segments there is no change after day 6. With S4, there may be a slight increase between days 4 and 5 but after this, dry weight remains more or less constant. Again with all four segments the terminal values between days 8 and 10 remain very similar, the maximum difference being about 10%.

The data of Series 1 show that while extension is proceeding in the cells of the leaf there is a sharp and sustained increase in dry weight. The data of Series 2 are consistent with this interpretation. In S2 and S3 while fresh weight per cell increases dry weight also increases. The data of Series 2 suggest that when extension growth has ceased dry weight increment decreases and soon stops also. The data, further, show that all the cells of the leaf tend to converge to similar mean volumes and dry weights. In the data of Series 1 it is evident that as fresh weight per cell increases dry weight increases and so also does the total nitrogen and protein content per cell. Furthermore, this increase in total nitrogen does not cease when the mature volume has been established. In the data of Series 2 the increase at 4 days from S1 to S2 to S3 in nitrogen content corresponds to the fact that there is also an increase in volume in this series.

The increase from S3 to S4 reflects the continued accumulation of nitrogenous components after extension has ceased. In S1 total nitrogen and protein increases from day 4 to day 5 to day 6 and there is a similar increase in S2. In S3 nitrogen accumulation ceases at day 5. Thus in these three segments there is no further accumulation of nitrogen after extension has ceased. The changes in S1, S2 and S3 correspond to the parallel changes in fresh weight and dry weight and are consistent with the conclusion that as extension continues total and protein nitrogen also increase. In S4 there is a significant increase in total and protein nitrogen between days 4 and 5. This does not correspond to any similar change in fresh weight or dry weight and after day 5 nitrogen content remains more or less constant.

Whereas with fresh weight and dry weight the terminal values are all more or less the same, with total and protein nitrogen they most certainly are not. The differences between the terminal values for S1 and S3 is of the order of 30% and between S1 and S4 it is of the order of 100%. In this connection, it is significant that while growth can occur in S1 after day 6 there is no further accumulation of nitrogenous compounds after this time. It has been shown that the order of values on the sixth day for fresh and dry weight per cell is a consequence of the relative degree of vacuolation at an earlier stage. In spite of the differences on the sixth day, however, all the curves for fresh and dry weight per cell tend to converge to the same approximate value on day 10. The data, therefore, suggest that after the sixth day, whereas the leaf can provide the components for dry weight increase, it cannot provide the components necessary for an increase in nitrogenous matter.

It may be inferred from these data that increment in total nitrogen per cell is some way dependent on an active meristem at the base of the leaf. The cells of S4, which completed their expansion while the meristem was fully active, have a considerably greater final nitrogen content than those of S1, S2 and S3. The cells of these basal segments expanded while the meristem was becoming progressively smaller and were found to have progressively lower final nitrogen contents. If the accumulation of nitrogen is dependent to some degree on the presence of an active meristem at the base of the leaf, then the mechanism involved is of some interest. If the process is a purely physical one then the meristematic tissue, being composed of non-vacuolate cells of high osmotic pressure, could accumulate nitrogen for the leaf through the process of diffusion. If the process is energy-requiring the meristem, considered as the basal segment, has a high rate of respiration and could, therefore, probably supply the energy required. However, it is also possible that with the development of the second and subsequent leaves, competition within the seedling for nitrogen becomes more severe. The first leaf, with its development almost complete and also being of smaller final size than the others, would provide a smaller 'sink' and would, therefore, be at a competitive disadvantage.

The initial and 4 day values for RNA content per cell in Series 2 (Fig.24) suggest that in 72 and 96 hr. leaves of Series 1 RNA content per cell would increase from the base to the apex. From the fourth to the fifth day, in Series 2, RNA per cell in S1, S2 and S3 increases, and between the fifth and sixth day there is a further sharp increase in S1 and S2. These changes support the conclusion that RNA increases as expansion proceeds.

A comparable decrease occurs in S1 and S2 between days 6 and 7. After the seventh day RNA continues to decline in all segments and reaches approximately the same value per cell on the ninth and tenth days. Thus it is probable that after the RNA content of the mature cell has been reached RNA decreases in contrast to the situation with dry and fresh weight and protein content. In terms of dry weight RNA is a comparatively small proportion of the total nitrogenous matter and the decrease in RNA is, therefore, unlikely to be reflected in the figures for total nitrogen.

The data for the ratio of RNA to protein (Fig.25) are of some interest in this connection. It is evident that on the third and fourth days the proportion of RNA is greatest in the dividing cells, the highest values being in S1 and S2. With the exception of the period of most rapid expansion, the proportion of RNA to protein decreases in all segments throughout the experimental period.

It was found by Rhodes and Yemm (1963) with intact first leaves of barley that, both in the light and the dark, there was an increase in RNA content per leaf to a peak value at about day 5 followed by a steady decline. Williams and Rijven (1965) with the fourth leaf of wheat found that the basal quarter of the leaf gave a peak RNA value around the time of cessation of growth of the leaf and that was followed by a decline. The top half of the leaf they found declined steadily in RNA content. Both these groups of workers found that protein content also rose to a peak but that this did not decline after the cessation of growth of the leaf in the light.

These results are fully consistent with those of the present work which allows their significance to be interpreted further. RNA content rises to a peak value with the progress of cell extension and then declines. When the meristem ceases to divide and its component cells extend, the leaf has its maximum cell content and also more cells in the process of extension than at any other time in the life of the leaf.

It is for this reason that peak values are found around the time of cessation of leaf growth in whole leaves.

With regard to DNA the sporadic data available suggest that while high average values per nucleus may be found in the meristematic region, due to the accumulation of DNA prior to division, once the 2C state has been restored after division and expansion has begun, there is no further change in DNA content during the experimental period.

This observation is also in agreement with the findings of Rhodes and Yemm (1963) and Williams and Rijven (1965). Using whole leaves these workers found that DNA content per leaf remained constant from approximately the time that division ceased in the basal meristem. It appears, therefore, that endopolyploidy does not occur in the cells of the leaves of wheat or barley. This contrasts with the frequent reports of endopolyploidy in roots (e.g. Heyes 1960, Sunderland and McLeish 1961, McLeish and Sunderland 1961) and also the report by Wright (1961) that DNA content continued to increase in the cells of the coleoptile of wheat after the mature volume had been reached.

The data of Series 1 show that chlorophyll content per cell (Fig.11) increases progressively from the base to the apex of the leaf both at 72 and at 96 hrs. Thus in the intact leaf although chlorophyll content per cell is increasing during expansion it continues to increase after expansion has ceased. The data of Fig.28 of Series 2 support this conclusion. In S1 chlorophyll content increases from day 3 to day 10, a similar change is shown by S2 and S3. In S4 there is a sharp increase from day 3 to day 4 and this might be expected from the data of Series 1. The increase in S4 continues, though at a far lower rate, after day 4.

On the tenth day, the values for chlorophyll content per cell are very different for the four segments, there being a threefold difference between the values for S1 and those for S4. On the other hand, the curves for S1, S2 and S3 are still rising and it is possible, though unlikely, that they could converge to the same value as S4 at a time later than 10 days.

The array of data relating to the quantitative characteristics of the average cell in different parts of the leaf indicate that expansion in all parts of the leaf leads to the establishment of approximately the same cellular volume in all parts. As expansion proceeds, dry weight increases but the dry weight may continue to increase for some time after the mature length has been reached. With extension there is an increase in total nitrogen and protein content and this continues until there is a change in the state of the leaf which prevents further accumulation after about the sixth day. As a result, the terminal protein and nitrogen values may differ considerably since on the sixth day when the change occurs, all cells have not reached the same protein and nitrogen content. Expansion is also accompanied by an increase in RNA; this increase may continue slightly after the mature length has been reached but it is evident that, at least in the later stages of the leaf, RNA content per cell falls as soon as the mature length has been reached. Further this fall is continuous to the end of the experimental period. Since protein content remains constant in the cells of each segment after day 6 the proportion of RNA to protein continues to decrease after the fourth day.

The changes in chlorophyll content per cell are apparently entirely unrelated to the changes in other components.

On both the third and fourth days chlorophyll content increases from the base to the apex of the leaf and it continues to increase in all cells of the system after the fourth day. This increase has not ceased by the end of the experimental period and the differences in chlorophyll content of the cells in the different regions of the leaf are still considerable at this time.

In the leaves of Series 1, on a unit cell basis (Fig.30), respiration rate is low in S1 and increases sharply from this to S3. At 72 hrs. the value recorded for S4 is slightly higher than that for S3. In the 96 hr. leaf it is probable that the value for S4 is slightly lower than that for S3. At 72 hrs., the volume of the cells increases from S1 to S4, at 96 hrs. it increases from S1 to about S5. In both sets of leaves, therefore, respiration is low in the meristematic cells and increases in the early stages of expansion. At 72 hrs., the rate remains constant from S3 to S5 and then decreases. At 96 hrs., the rate apparently remains constant from the fourth to the terminal segment. In the results of Series 2 (Fig.33) the rate for S1 increases from day 4 to day 5 and then further to day 6, this increase corresponds to the period when the cells are extending. In S2 there is a similar increase from day 4 to day 6 and this again is the period over which average volume per cell is increasing. In S3 there is little or no change from the fourth to the sixth day and it may be recalled that this corresponds to a situation where expansion has been completed from a comparatively late stage. After day 6 in S1, S2 and S3 there may be a steady decrease. This decrease is most marked in S2 and S3 in which extension is complete on the sixth day. In S4, which at 3 days corresponds to S5 of the 72 hr. leaf of Series 1 and at 4 days to S12 of the 96 hr. leaf, there is probably no change from 4 to 6 days but after this respiration rate decreases with time.

The terminal rates for S1, S2 and S3 are very similar but the rate for S4 is about 50% higher than that for S1. Thus the position seems to be that in the meristematic cell the rate is low and as expansion proceeds the rate tends to increase with time. However, before expansion is completed further increase in respiration rate ceases and the rate may then remain constant for a time or decrease. The data of Fig. 33 certainly suggest that in older leaves in all parts respiration tends to decrease progressively with time. The position with S4 in Series 2 requires some comment. As indicated earlier the high initial value in S4 at day 3 is probably due to a situation where the increase from S3 to S5 is more marked than the data in Fig.30 would suggest. The high relative value to S3 recorded on day 4 also probably represents a situation where S12 of the 96 hr. leaf of Series 1 has a slightly higher respiration rate than that shown in Fig.30. Subsequent to the sixth day, although respiration rate declines as in other segments, it remains higher than the rest. This is probably connected with the fact that this segment has a higher protein content at all stages of development and also contains more catalytically active protein with regard to respiration. The data showing respiration per unit protein in Series 1 and Series 2 are of some importance. In the 72 and 96 hr. leaves of Series 1 (Fig.30) respiration is relatively low in S1 and increases from this to S2 and S3. Thereafter with both leaves it decreases with increasing distance from the base of the leaf. The values for the 72 hr. leaf are shown to be generally higher per unit protein than those for the 96 hr. leaf. It may be noted that since respiration falls sharply per unit protein after S3 the rate in S5 at 72 hrs. and in S12 at 96 hrs. are considerably lower than the values for S2 and S3.

The data per unit protein for Series 2 (Fig.34) reproduce some of the relative differences shown in Series 1. S4, which corresponds to S5 at 72 hrs. and S12 at 96 hrs., has consistently the lowest respiration. The highest rate is given by S2 and S3 with S1 in an intermediate position. With all segments there is a drop between the third and fourth days, this corresponds to the situation in the 72 hr. leaf where the basal values are higher than those of the 96 hr. leaf. In S1, S2 and S3 respiration increases between the fourth and fifth days as extension proceeds in these segments. With S1 and S2 a decrease begins on the sixth day and in S3 on the fifth day. These decreases continue to the end of the experimental period. This is clearly a continuation of the trend shown in Series 1 of a decrease in respiration as cells age after expansion.

The whole group of results seem to suggest that in the leaf respiration is low in the meristematic cells and increases as the cells expand. This increase, however, ceases before the maximum size is reached and thereafter remains more or less constant or decreases. The specific activity per unit protein is again relatively low in the meristematic cell, increases as the cell expands, but reaches a maximum before the mature volume is reached. After the maximum respiration rate is reached, specific activity decreases progressively with time. In S1, S2 and S3 the terminal respiration rate per unit protein appears to be very similar but in S4 it is considerably lower than in the other three. It is in S4 that the highest protein content per cell is recorded and the markedly lower specific activities here suggest that much of this protein is inert with respect to respiration. However, since S4 has a higher rate per cell than the other segments, clearly some of the additional protein must be active with regard to respiration.

The results of Series 1 show that in the 72 and 96 hr. leaves, invertase activity per cell (Fig.30) is low in S1 and increases from this to S4, thereafter it decreases steadily in both leaves with increasing distance from the base. The data suggest that the increase is greater in the 96 hr. leaf than in the 72 hr. one, all the values after S4 being greater at 96 hrs. than at 72. These results indicate that with expansion there is an increase in invertase activity per cell, this increase reaches a peak at about the point where the maximum length is reached and thereafter activity declines with increasing age of the cells. The results in Series 2 indicate the same conclusion (Fig.36), at day 4 the highest value is given by S3 with S4 next and then S2 and S1 lowest. S1 contains meristematic cells, in S2 vacuolation has started and in S3 it has progressed further. S4 corresponds to S12 of the 96 hr. leaf and the relatively low values given by this segment accord with the low apical values in the 96 hr. leaf. In S1 invertase activity increases sharply after the fifth day and this increase continues up to the seventh day. In this segment, expansion was particularly rapid between day 5 and day 6 and does, in fact, continue up to day 7. In S2 there is about a tenfold increase in invertase activity per cell between the fourth and the sixth day. In S3 again activity increases greatly between day 4 and day 6; in both these segments the average cell volume is increasing greatly over this interval. With S1 activity decreases after day 7 and with S2 and S3 it decreases progressively with time after day 6. The changes in S4 after the fourth day may be anomalous since this segment also shows a peak at day 6, however, compared with the value for S2 that for S4 is relatively small. After day 6, the values for S4 decline steadily.

In view of the very large differences that occur between segments in the early part of the experimental period the differences on the tenth day are relatively small and it is possible that all segments converge to the same value. In general, therefore, the results of Fig.36 confirm those of Fig.30 in showing that there is a very large increase in invertase activity during expansion, and that this is followed by a decrease once the mature volume has been attained.

The changes in invertase activity per unit protein (Fig.31) for Series 1 show that in the 72 hr. leaf the lowest specific activity is found in the meristematic cells, this increases to S3 and then steadily decreases. A similar situation is shown with the 96 hr. leaf except that here the increase continues to S4. Thus as expansion proceeds not only does total activity increase but also specific activity. It is probable that a peak is reached before the maximum volume is established. After the peak value, specific activity decreases with distance from the base. In the data for Series 2 (Fig.37) the specific activity at day 4 is lowest in S1 and highest in S3 with S2 intermediate, S4 gives a low value corresponding to the low value of S12 at 96 hrs. In S1 specific activity increases slightly from day 4 to day 5 and sharply from day 5 to day 7. The period between day 5 and day 7 being that in which volume per cell increases sharply in this segment. Between the fourth and the sixth day there is a sharp increase in specific activity in S2 and S3, this again coincides with the principal period of cell expansion for these segments. After day 7 in S1 and after day 6 in S2 and S3 specific activity declines steadily with time. In S4 specific activity decreases steadily with time throughout the experimental period.

It is significant that the apparent increase per cell between days 4 and 6 is not shown on a unit protein basis.

It is clear that expansion is accompanied by an increase in invertase activity and part of this increase can no doubt be referred to the increase in protein which occurs over the same period but a large part of it is undoubtedly due to a changing pattern in the catalytic activity of the protein as is shown by the increasing specific activity during expansion. When expansion is complete protein content does not change but invertase activity per cell decreases. Thus the decrease in invertase activity per cell must be solely due to a change in the catalytic activity of the protein.

The data for phosphatase activity per cell in Series 1 (Fig.30) show that at 72 hrs. activity is lowest in S1 but increases sharply from here to S3 and may increase still further to S4. After S4 it is probable that activity is more or less constant. With the 96 hr. leaf, activity is again lowest in S1 and here it increases to S5 and thereafter remains more or less constant. The low value for S1 in the 72 and 96 hr. indicates that per cell phosphatase activity is lowest in the meristematic tissue and increases over the early period of expansion. The rate of activity in the mature segments remains more or less constant.

The phosphatase activities for Series 2 are shown in Fig.39. Activity is in the order S1 to S2 to S3 to S4 at days 3 and 4 and this is also the order of increasing mean cell volume. In S1, activity per cell increases sharply from day 4 to day 6 and in S2 and S3 from day 4 to day 5. In all three segments the phase of increase corresponds to the early, rapid phase of expansion.

The more prolonged period of expansion in S1 corresponds to the longer period over which activity is increasing in this segment. After peak activity is reached in each of these segments, there may be a slight decrease in activity per cell with time. In S4 there is a sharp increase in activity from day 4 to day 5, but a sharper decrease after day 5 than in other segments. In this segment there is no correlation of increasing activity with the phase of expansion. The general array of data on activity per cell suggests that as expansion proceeds there is an increase in phosphatase activity which may reach a peak before expansion is complete and then decline slightly with time. The special case of S4 must be interpreted in terms of increasing protein content and this is referred to again below.

The values for phosphatase activity per unit protein in Series 1 (Fig.31) show that in the 72 hr. leaf there is an increase from S1 to S2 and then a slow decrease to S7. With the 96 hr. leaf there is a slight increase from S1 to S5 and a slight decrease thereafter. These results suggest that the initial rise in activity per cell is related primarily to the increase in the total amount of protein and not to a change in the proportion of enzyme in the protein. The subsequent decrease in specific activity is again a consequence of a rise in protein content; since this compensates for the drop in specific activity, the activity per cell remains constant. In Series 2 (Fig.40) specific activity on day 4 is more or less the same in all segments. There is a sharp rise from day 4 to day 6 in S1 and from day 4 to day 5 in S2 and S3. The rise to day 6 in S1 corresponds to the increasing volume of the cells in this segment although it is significant that the increase ceases before the maximum volume is reached.

In S2 and S3 the increase ceases on the fifth day which again precedes the achievement of maximum volume. After day 6 with S1 and after day 5 with S2 and S3 there is a slow decrease in activity. With S4 specific activity probably decreases progressively throughout the experimental period. The general trends shown by these data are clearly similar to those of the corresponding data in Series 1. Increasing activity per cell during the phase of expansion is primarily a consequence of increasing content of protein. The slow decrease per cell is evidently caused by a similar change in the specific activity of the protein. It is clear that the rise per cell from day 4 to day 5 in S4 is simply a consequence of increasing protein content which carries a proportion of the enzyme since specific activity does not show a significant rise during this interval.

The changes in protease activity per cell in Series 1 (Fig.30) show that in the 72 hr. leaf protease activity increases from S1 to S3. The data available indicate that after S3 activity levels off. This is not in accord with the results in the 96 hr. leaf or with those of Series 2. The data for the 96 hr. leaf show that activity is low in the meristem, S1, increases sharply to S4 and then continues to increase slowly with distance from the base of the leaf. The results of Series 1, therefore, indicate that protease activity increases during expansion but that the increase does not cease when the maximum volume is reached but continues to increase as the tissue gets older. The data per cell for Series 2 (Fig.42) show that for day 3 the order of increasing activity is from S1 to S2 to S3 to S4 which suggests that in the 72 hr. leaf of Series 1 protease activity really increases throughout the leaf from base to apex.

The order on the fourth day also increases from S1 to S4 which corresponds to the values found for the 96 hr. leaf in Series 1. In S1 of Series 2 after day 4, there is a pronounced rise to day 6 and a continuous slower rise thereafter. With S2 there is again a rise from the fourth to the sixth day corresponding to a period of rapidly increasing cell volume. In S3 there is a rise from the fourth to the fifth day which covers part of the period of increasing volume. With S2 and S3 there may be a slight decrease in activity after the peak value has been reached. With S4 there is a rise from day 4 to day 5 and it is probable that there is a slow decrease thereafter.

The data for specific activity for Series 1 (Fig.31) show that at 72 hrs. there is little or no change in specific activity from S1 to S3 but the subsequent values show a sharp decrease. For reasons given below these latter values are considered unreliable. In the 96 hr. leaf it is probable that there is little or no change between S1 and S6 although there may be a slight decrease in specific activity thereafter. The specific activity values for Series 2 (Fig.43) suggest that there is little change after day 4. With S1 and S2 there may be a slight increase from day 4 to day 5 but this is not very pronounced. All data except those for S1 suggest that a slow decrease with time occurs which is most pronounced after day 6. The data indicate that during the first phase of expansion the proportion of the enzyme in the total protein does not change significantly and the increase in activity per cell during this period is due solely to an increase in the total mass of protein. The increase per cell in the 96 hr. leaf of Series 1 from S4 to the apex must also be attributed to increasing quantity of protein since specific activity does not increase over this region.

The rise in activity per cell in Series 2 from day 4 to day 6 in S1, S2 and S3 must be attributed directly to increasing protein content and the decline after day 6 must be due to a corresponding decrease in specific activity. It is significant that with S4 there is no change in specific activity from day 3 to day 6 and the increase in activity per cell over this period is again a consequence of increasing mass of protein which carries the same proportion of the enzyme. However, the reduction in activity per cell after day 6 in S4 is clearly related to a decrease in specific activity.

Data are not available on changes in photosynthetic activity in Series 1. The data of Fig.45 are for Series 2 and show photosynthetic activity in different parts of the leaf at different times. These suggest that on the third day, and thus in the 72 hr. leaf, activity is lowest in the basal segment and increases with increasing distance from the base. A similar situation is suggested by the data for day 4. The changes after day 4 do not indicate any marked changes with growth in any of the segments. In S1 there may be a slight increase with time and a similar rise in S2, in S3 values remain constant after day 4 as they also do for S4. It is probable that a different pattern would have appeared if a higher light intensity had been used. It is doubtful, however, if the order of activity in the different segments would have changed and it is the relative order that is the most significant feature of these data. It is clear that the relative order is not determined by chlorophyll contents. Fig.46 gives data showing photosynthesis per unit chlorophyll and these values show that the lowest rate in these terms is given by the most mature segment and the highest by the basal segment. On the other hand, the highest absolute value per cell is found in S4 and the lowest in S1, this suggests that the order in photosynthetic rate per cell is more closely related to total protein than to chlorophyll content.

It may be presumed that the amount of chloroplast protein is the limiting factor in controlling photosynthetic rate. S4 has the highest protein content and presumably, therefore, the highest content of chloroplast protein. The protein content per cell decreases from S3 to S2 to S1 and so does the order of photosynthetic activity. The data suggest that chlorophyll formation is independent of chloroplast protein formation and that since it is probably these proteins that carry the enzymes for the light and dark reactions of photosynthesis they are the ultimate controlling factor in photosynthetic rate. Gabrielsen (1948) studied the efficiency of light energy conversion in relation to chlorophyll concentration. He found that after a certain concentration no further increase in photosynthesis occurred with additional chlorophyll however great the light intensity provided. It appears that the accumulation of chlorophyll by plant leaves is not related to the maximum rate of photosynthesis of which they are capable.

The pattern of events involved in the later stages of the growth of the leaf, particularly with regard to expansion may now be summarised. On about the third day, the cells are in an age series as the leaf is traversed from base to apex. At this time, meristematic activity is intense in the basal intercalary meristem, the situation is very similar 24 hrs. later with active division still proceeding and the cells still in an age series. The major zone of expansion stretches from the meristem to about 2 cm. from the base of the leaf. After day 4, meristematic activity begins to decrease and ceases after day 6. Any increase in the length of the leaf after this time is, therefore, solely due to expansion.

Cells which are in an intermediate stage of expansion complete this process and cells which were previously meristematic start expanding at different times and thus promote a progressive shrinking of the meristem. It is this progressive erosion of the meristem which accounts for expansion proceeding for at least three days after division has begun decline in the base of the leaf.

The dry weight of the cells increases as expansion proceeds and this increase does not cease when the mature length has been reached, but continues throughout the whole period covered by the present series of experiments. However, the increase during the latter part of the period is slight and may not be significant. The increase in dry weight is due, principally, to an accumulation of polysaccharide material in the cell walls. The proportion of the dry weight due to nitrogenous matter falls progressively with time; it starts at a value of about 70% and decreases to about 17% at the end of the experimental period in some parts of the leaf. The development of the cell is, however, accompanied by an absolute increase in protein. Protein increases as extension proceeds and again the accumulation of protein does not necessarily cease when the cell has reached the mature length, it may continue for about 24 hrs. after this but then apparently remains more or less constant for the final four or five days of the experimental period.

With the increase in protein during expansion there is also an increase in RNA: it appears that, unlike ~~with~~ protein, RNA, content falls as soon as the mature length is reached and continues to do so until the end of the experimental period.

Throughout the development of the cells, chlorophyll content increases. Some chlorophyll is present in the meristematic tissue and it increases in all cells throughout the experimental period. There is no indication that change in chlorophyll is related to either the process of expansion or to the time at which any particular part of the leaf emerges from the coleoptile. The data indicate that light intensity has little or no limiting effect on the formation of chlorophyll. Sufficient light is able to reach the developing cells when they are within the coleoptile to ensure the progressive accumulation of chlorophyll. It should be noted that in the leaves of Series 2 accumulation of chlorophyll was faster than in the leaves of Series 1, but it will be remembered that the material of Series 2 was grown at a longer daylength as well as a higher light intensity than that of Series 1.

The composition of the protein that is accumulated both during and after expansion evidently changes and continues to change after maturity has been reached, though the total quantity of protein at maturity remains constant. There is some indication that the proportion of chloroplast protein also changes and that the photosynthetic capacity of the tissue is controlled by this condition.

The change in the total amount of protein per cell implies corresponding changes in metabolic activity. During the major phase of expansion there is an increase in respiration and in the activities of various enzyme systems. The data available indicate that during expansion there is a sharp increase in respiration which thereafter remains more or less constant per cell.

The relative increase in respiration, however, is less than the relative increase in protein so during the expansion phase respiration per unit protein tends to decrease. Since protein content tends to increase or remain constant after extension respiration per unit protein continues to decrease after the maximum length has been reached, to the end of the experimental period. There are no critical data available on possible substrate changes which could account for the relative change of respiration rate with time. It is significant that during measurement in the Warburg apparatus over a two hour period the rates always remained constant and it is, therefore, probable that the relative changes are due to corresponding changes in the proportion of the respiratory enzyme proteins in the total protein.

The determinations made on individual enzymes support this conclusion. With increase in cell volume there is an increase in invertase activity, the peak here being reached at the same time as maximum length, and then a decline to the end of the experimental period. When the invertase activities are expressed per unit protein there is still a sharp increase in activity during the period of expansion and sharp decline thereafter. The data, therefore, indicate that the proportion of invertase enzyme in the total protein is increasing during the phase of extension and decreasing subsequently.

With protease activity there is also an increase during the phase of expansion and here the increase continues after the maximum length has been reached. When protease activity is expressed on a unit protein basis there is still an increase during the phase of extension but subsequently a slow decrease: this suggests that during the phase of extension the proportion of protease enzyme in the total protein is increasing and thereafter slowly decreasing.

With phosphatase activity the changes are less sharp. There is an increase per cell during extension and after the maximum length has been reached, activity either remains constant or decreases slightly. On a per unit protein basis there is little change during the period of expansion or after it. In this case, evidently, the proportion of phosphatase enzyme in the total protein remains more or less constant and changes in activity on a per cell basis are due primarily to changes in the total amount of protein.

These results may be compared with similar results obtained using similar techniques in the root (Brown and Broadbent 1950, Robinson and Brown 1952 and 1954, Robinson 1956). After the mature length has been reached, cell wall weight continues to increase in both leaf and root but whereas in the root protein content tends to decrease after this point, in the leaf it does not, indeed it continues to increase. RNA increases in both systems as cell volume increases and then decreases, this decrease appears to start somewhat earlier in the leaf than in the root. In the root endopolyploidy occurs in the period of maturity with a consequent increasing average DNA content but in the leaf the indications are that it remains constant at the 2C value in all cells. The progress of expansion is accompanied by corresponding changes in metabolic activity; respiration, invertase, phosphatase and protease increase in activity in both systems, root and leaf. In both also there is an increase in invertase and protease activity relative to protein and in both again there is little relative change in phosphatase activity. But, whereas in the root respiration increases relative to protein in the leaf it does not, on the contrary it decreases.

When the mature volume has been reached in both root and leaf invertase activity decreases but whereas in the root respiration, phosphatase activity and protease activity also tend to decrease in the leaf they either slightly increase or remain more or less constant. On a unit protein basis in the leaf and the root respiration and invertase activity decrease after extension and in both also there is little change in phosphatase activity over this period. However, in the root relative protease activity decreases sharply while in the leaf it does not.

The development of the cells of the wheat leaf, as investigated by the serial segment technique, show many similarities and a few differences from those of the root. In the root, all the cells cut off from the apical meristem are found to follow a similar developmental pattern. This is also true for the gramineous leaf but here the meristem is basal and functions for a limited time. The cells which extend as a result of the cessation of divisional activity in the meristem develop somewhat differently from those cut off earlier from an active meristem.

Results of this investigation support the findings in the root which were used by Heyes and Brown (1965) for their hypotheses on cell differentiation. They suggest that in the meristematic cell, expansion and differentiation cannot occur unless there is an alteration in the protein "state" of the cells. This also requires an alteration in the enzymic content and catalytic activity of the cells.

Once expansion has started, the protein "state" constantly alters with the concomitant changes in total enzyme activity and in the ratio of the activity of one enzyme to that of another. Without this changing metabolic pattern, differentiation could not occur. Furthermore, these changes do not stop when expansion is complete, but continue throughout the life of the cell.

The present work emphasises the limited significance that can be placed on the results of work using whole leaves when such results are placed on a unit cell basis.

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APPENDIX I

TABLE OF CONTENTS

DATA FOR SERIES I

DATA FOR SERIES II

DATA FOR SERIES I

The tables below give the values for cell number per segment together with the standard error. The values given are the means of six determinations.

	72 hr. leaves	
	Cell number per segment	S.E.
1.	210,300	6,500
2.	86,600	3,200
3.	41,300	400
4.	34,800	500
5.	33,300	1,200
6.	32,200	1,000
7.	24,600	600
	96 hr. leaves	
1.	211,300	5,400
2.	138,100	2,800
3.	47,200	1,200
4.	36,000	800
5.	32,200	700
6.	31,600	900
7.	31,300	700
8.	30,600	400
9.	29,700	600
10.	28,400	500
11.	26,300	1,000
12.	26,300	900
13.	21,500	400
14.	16,600	300

The tables below give the value for fresh weight per segment and per cell, and for dry weight per segment and per cell. The fresh weight figures are the means of seven determinations and the dry weight figures are the means of three determinations.

	72 hr. leaves					
	Fresh Weight			Dry Weight		
	Per Segment mgs.	S.E.	Per cell mgs. x 10^5	Per segment mgs.	S.E.	Per cell mgs. x 10^5
1.	1.45	0.08	0.69	0.25	0.009	0.119
2.	1.65	0.08	1.91	0.25	0.008	0.289
3.	1.86	0.10	4.51	0.22	0.010	0.533
4.	1.90	0.10	5.46	0.27	0.008	0.775
5.	1.91	0.07	5.74	0.30	0.008	0.902
6.	1.82	0.07	5.66	0.29	0.010	0.901
7.	1.34	0.03	5.44	0.25	0.007	1.016
	96 hr. leaves					
1.	1.26	0.06	0.60	0.26	0.008	0.123
2.	1.44	0.08	1.04	0.24	0.008	0.174
3.	1.54	0.08	3.26	0.24	0.006	0.509
4.	1.61	0.04	4.48	0.26	0.006	0.724
5.	1.72	0.08	5.34	0.29	0.007	0.901
6.	1.79	0.07	5.67	0.30	0.010	0.951
7.	1.79	0.07	5.73	0.28	0.010	0.896
8.	1.76	0.10	5.75	0.31	0.012	1.012
9.	1.74	0.05	5.86	0.29	0.009	0.977
10.	1.71	0.10	6.01	0.30	0.014	1.055
11.	1.71	0.08	6.51	0.32	0.013	1.219
12.	1.70	0.06	6.78	0.31	0.013	1.181
13.	1.53	0.04	7.11	0.28	0.008	1.301
14.	0.96	0.04	5.80	0.24	0.006	1.449

The tables below give the values for total, TCA soluble and TCA insoluble nitrogen on a per segment basis. The values for total nitrogen are the mean of four separate estimations and the values for soluble and insoluble nitrogen are the means of three estimations.

Tables of Nitrogen Content per Segment

Segment	Total Nitrogen		TCA Soluble Nitrogen		TCA Insoluble Nitrogen		Protein
	µgs.	S.E.	µgs.	S.E.	µgs.	S.E.	µgs.
72 hr. leaves							
1	30.5	1.6	4.2	0.41	25.6	1.6	160.0
2	19.4	1.2	3.6	0.22	16.5	1.3	103.1
3	14.8	1.0	2.4	0.18	12.9	1.0	80.6
4	14.9	1.3	2.4	0.16	12.9	0.8	80.6
5	15.0	1.2	2.6	0.20	13.1	0.9	81.9
6	15.2	0.9	2.8	0.20	13.3	0.9	83.1
7	12.7	0.7	2.1	0.14	10.5	0.6	65.6
96 hr. leaves							
1	31.8	1.7	5.2	0.40	25.4	1.5	158.8
2	26.4	1.5	3.7	0.26	21.5	1.4	134.4
3	19.0	1.3	2.3	0.17	14.5	1.2	90.6
4	15.4	1.2	1.8	0.18	10.7	1.0	66.9
5	14.2	1.2	2.4	0.20	9.8	1.0	61.3
6	14.8	1.3	2.5	0.18	11.2	0.9	70.0
7	15.4	1.3	2.2	0.17	12.3	0.9	76.9
8	16.0	1.4	1.9	0.19	12.2	0.8	76.3
9	16.4	1.2	2.0	0.14	11.8	0.9	73.8
10	16.8	1.4	2.7	0.17	12.3	1.0	76.9
11	17.2	1.3	1.6	0.16	13.4	0.9	83.8
12	17.4	1.2	1.4	0.18	14.7	0.8	91.9
13	16.8	1.1	1.6	0.12	12.8	0.9	80.0
14	12.8	0.8	1.0	0.09	10.4	0.5	65.0

The protein values were obtained by multiplying the figures for insoluble nitrogen by the statutory factor of 6.25.

The tables below give the values for total nitrogen per cell, the weight of nitrogenous matter per cell and the percentage of nitrogenous matter in the total dry weight.

	Total Nitrogen per cell mgs. x 10^{7}	72 hr. leaves Total Nitrogenous matter per cell mgs. x 10^{5}	Percentage Nitrogenous matter in total dry weight
1.	1.45	0.091	76.2
2.	2.24	0.140	48.5
3.	3.59	0.224	41.9
4.	4.28	0.267	34.5
5.	4.51	0.282	31.3
6.	4.73	0.295	32.8
7.	5.16	0.322	31.7
		96 hr. leaves	
1.	1.51	0.094	76.4
2.	1.91	0.119	68.7
3.	4.03	0.252	49.5
4.	4.29	0.268	37.0
5.	4.41	0.276	30.6
6.	4.69	0.293	30.8
7.	4.93	0.308	34.4
8.	5.22	0.327	32.3
9.	5.52	0.345	35.3
10.	5.91	0.369	35.0
11.	6.55	0.409	33.6
12.	6.63	0.414	35.1
13.	7.81	0.488	37.5
14.	7.73	0.433	29.9

The tables below give the values for chlorophyll a, chlorophyll b and total chlorophyll per segment, and total chlorophyll per cell. The ratio of chlorophyll a (Ca) to chlorophyll b (Cb) is also given, as is the standard error for total chlorophyll.

	Ca per segment mgs. x 10^4	Cb per segment mgs. x 10^4	Ratio Ca/Cb	Total C per segment mgs. x 10^4	S.E.	Total C per cell mgs. x 10^8
			72 hr. leaves			
1.	1.01	0.50	2.0	1.51	0.14	0.07
2.	2.69	0.82	3.3	3.51	0.18	0.41
3.	3.07	1.00	3.1	4.08	0.29	0.99
4.	3.33	0.99	3.4	4.32	0.33	1.24
5.	4.15	1.05	3.9	5.18	0.60	1.56
6.	5.38	1.34	4.0	6.72	0.76	2.09
7.	5.79	1.72	3.4	7.51	0.57	3.05
			96 hr. leaves			
1.	1.48	0.83	1.8	2.30	0.21	0.11
2.	3.24	0.97	3.3	4.21	0.46	0.31
3.	3.82	1.28	3.0	5.09	0.75	1.08
4.	3.89	1.37	2.8	5.26	0.81	1.46
5.	4.27	1.32	3.2	5.59	0.90	1.74
6.	4.86	1.48	3.3	6.34	0.86	2.01
7.	5.78	1.73	3.3	7.50	0.91	2.40
8.	6.49	1.87	3.5	8.33	0.96	2.72
9.	8.13	2.23	3.6	10.33	0.71	3.48
10.	9.45	2.68	3.5	12.13	0.66	4.27
11.	10.71	3.08	3.5	13.75	0.19	5.24
12.	11.80	3.42	3.4	15.23	0.55	5.80
13.	12.28	3.66	3.3	15.95	0.29	7.41
14.	8.48	2.76	3.1	11.26	0.74	6.80

The tables below give the values for respiration on a per segment, per cell and per µg. protein basis. A table of standard error for the primary data is also given. The results are expressed as µ litres of oxygen evolved.

	72 hr. leaves			
	µl O_2 per segment per hr.	S.E.	µl O_2 per cell per hr. x 10^5	µl O_2 per µg. protein per hr. x 10^2
1.	2.95	0.34	1.40	1.84
2.	2.87	0.30	3.32	2.78
3.	2.17	0.26	5.26	2.69
4.	1.84	0.21	5.28	2.29
5.	1.77	0.20	5.32	2.16
6.	1.64	0.17	5.10	1.97
7.	1.07	0.15	4.35	1.63
			96 hr. leaves	
1.	2.84	0.28	1.34	1.79
2.	3.03	0.31	2.19	2.25
3.	2.42	0.20	5.13	2.67
4.	1.65	0.18	4.59	2.47
5.	1.58	0.19	4.91	2.58
6.	1.46	0.16	4.63	2.09
7.	1.42	0.16	4.54	1.85
8.	1.48	0.14	4.83	1.86
9.	1.40	0.18	4.72	1.90
10.	1.35	0.17	4.75	1.76
11.	1.28	0.14	4.88	1.53
12.	1.34	0.15	5.10	1.46
13.	1.10	0.13	5.11	1.38
14.	0.86	0.09	5.19	1.32

The Tables below give the values for invertase activity on a per segment, per cell and per µg. protein basis. A table of standard error for the primary data is also given. The results are expressed as µ moles of reducing sugar (µ M.r.s.) produced.

	µ M.r.s. per segment per hr.	S.E.	µ M.r.s. per cell per hr. x 10^4	µ M.r.s. per µg. protein per hr. x 10^2
		72 hr. leaves		
1.	3.56	0.42	0.17	2.23
2.	8.33	0.61	0.96	8.08
3.	11.70	1.10	2.84	14.52
4.	10.46	1.08	3.00	12.98
5.	9.13	0.93	2.74	11.15
6.	8.42	0.71	2.62	10.13
7.	5.71	0.30	2.32	8.70
		96 hr. leaves		
1.	3.53	0.32	0.17	2.22
2.	8.50	0.47	0.62	6.32
3.	11.43	1.20	2.42	12.62
4.	11.25	1.14	3.12	16.79
5.	9.72	1.04	3.02	15.87
6.	8.87	0.82	2.81	12.67
7.	6.37	0.66	2.04	8.28
8.	6.21	0.64	2.03	8.14
9.	5.99	0.75	2.02	8.12
10.	5.28	0.50	1.86	6.87
11.	5.19	0.51	1.98	6.19
12.	4.41	0.34	1.68	4.80
13.	3.07	0.42	1.43	3.84
14.	1.13	0.34	0.68	1.74

The Tables below give the values for protease activity on a per segment, per cell and per µg. protein basis. A table of standard error for the primary data is also given. The results are expressed as µgs. of tyrosine equivalents produced.

	µgs. tyrosine per segment per hr.	S.E.	µgs. tyrosine per cell per hr. x 10^4	µgs. tyrosine per µg. protein per hr. x 10^2
		72 hr. leaves		
1.	20.8	2.14	0.99	13.00
2.	13.4	1.52	1.55	13.00
3.	11.4	1.26	2.76	14.14
4.	8.0	1.18	2.30	9.93
5.	9.5	1.35	2.36	11.60
6.	8.0	1.04	2.49	9.63
7.	4.8	0.62	1.93	7.32
		96 hr. leaves		
1.	21.9	2.23	1.04	13.79
2.	19.8	1.74	1.43	14.73
3.	12.0	1.08	2.04	13.25
4.	11.4	1.02	3.17	17.04
5.	10.8	0.85	3.34	17.62
6.	11.8	0.74	3.72	16.86
7.	11.8	0.72	3.76	15.34
8.	12.4	1.01	4.05	16.25
9.	12.5	0.93	4.21	16.94
10.	12.6	0.76	4.45	16.38
11.	13.0	0.66	4.95	15.51
12.	13.8	0.61	5.24	15.02
13.	11.2	0.47	5.18	14.00
14.	8.8	0.33	5.28	13.54

The Tables below give the values for phosphatase activity on a per segment, per cell and per µg. protein basis. A table of standard error for the primary data is also given. The results are expressed as µgs. of inorganic phosphorous produced.

	µg. Pi per segment per hr.	S.E.	µg. Pi per cell per hr. x 10^4	µg. Pi per ug. protein per hr. x 10^2
		72 hr. leaves		
1.	10.90	1.61	0.52	6.81
2.	9.80	1.22	1.13	9.51
3.	7.04	0.97	1.71	8.73
4.	6.10	0.75	1.75	7.57
5.	5.50	0.70	1.65	6.62
6.	5.10	0.62	1.59	6.14
7.	4.36	0.34	1.77	6.65
		96 hr. leaves		
1.	6.30	1.84	0.30	3.97
2.	8.18	1.16	0.59	6.09
3.	6.00	0.96	1.27	6.62
4.	5.33	0.87	1.48	7.97
5.	5.94	0.80	1.85	9.69
6.	5.29	0.72	1.68	7.56
7.	5.27	0.64	1.69	6.85
8.	5.22	0.66	1.71	6.84
9.	5.25	0.72	1.77	7.11
10.	4.69	0.54	1.65	6.10
11.	4.77	0.40	1.82	5.69
12.	4.75	0.42	1.81	5.17
13.	4.27	0.41	1.98	5.34
14.	3.20	0.28	1.93	4.92

DATA FOR SERIES II

The table below gives the values for cell number per segment and also for standard error.

	S1		S2		S3		S4	
	No.	S.E.	No.	S.E.	No.	S.E.	No.	S.E.
Day 3	215,600	5,200	114,400	4,300	53,800	2,800	32,500	400
4	227,500	6,100	131,300	2,700	52,100	2,000	30,300	600
5	154,400	3,000	79,400	1,400	38,400	1,200	30,000	800
6	47,500	1,200	32,200	300	31,200	400	30,600	700
7	39,100	600	31,900	600	31,900	400	31,200	500
8	34,400	400	32,200	500	31,200	300	31,600	400
9	32,500	600	31,200	500	32,200	500	30,900	600
10	32,800	700	31,900	600	31,200	400	30,600	600

The tables below give the values for fresh weight per segment and per cell and also the standard error for the primary data. The values are the means of five determinations.

Fresh Weight per Segment mgs.

	Segment 1	S.E.	Segment 2	S.E.	Segment 3	S.E.	Segment 4	S.E.
Day 3	1.821	0.07	2.107	0.09	2.033	0.09	2.351	0.11
4	2.048	0.08	2.339	0.10	2.393	0.09	2.711	0.14
5	2.510	0.12	2.470	0.12	2.559	0.13	2.931	0.14
6	2.565	0.12	2.464	0.11	2.578	0.13	2.925	0.15
7	2.876	0.13	2.563	0.13	2.674	0.14	2.922	0.16
8	2.841	0.10	2.606	0.13	2.743	0.12	2.895	0.13
9	2.770	0.12	2.638	0.14	2.854	0.15	2.945	0.15
10	2.712	0.11	2.610	0.12	2.752	0.14	2.899	0.14

Fresh Weight per Cell mgs. x 10^5

	Segment 1	Segment 2	Segment 3	Segment 4
Day 3	0.845	1.842	3.782	7.234
4	0.900	1.782	4.909	9.670
5	1.626	3.112	6.658	9.770
6	5.400	7.655	8.266	9.551
7	7.363	8.041	8.389	9.350
8	8.265	8.096	8.778	9.172
9	8.523	8.442	8.867	9.519
10	8.265	8.188	8.806	9.466

The tables below give the values for dry weight per segment and per cell, and also the standard error for the primary data. The values are the means of five determinations.

Dry Weight per Segment in mgs.

	S1	S.E.	S2	S.E.	S3	S.E.	S4	S.E.
Day 3	0.278	0.012	0.244	0.009	0.224	0.007	0.304	0.006
4	0.278	0.014	0.257	0.012	0.247	0.009	0.318	0.007
5	0.286	0.014	0.262	0.011	0.261	0.009	0.350	0.007
6	0.341	0.016	0.318	0.014	0.331	0.012	0.362	0.008
7	0.362	0.017	0.357	0.013	0.349	0.010	0.377	0.007
8	0.370	0.017	0.364	0.014	0.361	0.013	0.379	0.006
9	0.368	0.019	0.372	0.015	0.380	0.014	0.388	0.009
10	0.376	0.016	0.369	0.013	0.387	0.014	0.383	0.008

Dry Weight per Cell in mgs. x 10^6

	S1	S2	S3	S4
Day 3	1.289	2.133	4.167	9.354
4	1.222	1.958	4.739	10.491
5	1.853	3.301	6.779	11.667
6	7.179	9.860	10.344	11.820
7	9.267	11.200	10.942	12.064
8	10.764	11.309	11.552	12.000
9	11.323	11.904	11.783	12.516
10	11.481	11.576	12.384	12.506

The table below gives the values for the percentage of nitrogenous matter in the total dry weight.

	S1	S2	S3	S4
Day 3	57.5	46.1	35.7	28.4
4	70.1	58.1	43.1	30.1
5	53.1	44.9	39.5	40.9
6	29.7	26.4	26.2	39.4
7	22.7	21.6	23.8	38.6
8	19.0	20.7	23.0	40.0
9	17.7	19.0	22.5	36.7
10	17.1	19.4	22.6	38.2

The tables below give the values for T.C.A. soluble, T.C.A. insoluble, and total nitrogen per segment in µgs. A table of standard error for total nitrogen is also given.

Table of Soluble and Insoluble Nitrogen per Segment in µgs.

	S1		S2		S3		S4	
	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble	Soluble	Insoluble
Day 3	5.4	20.2	3.9	14.1	3.0	9.8	2.8	11.0
4	5.8	25.4	4.8	19.1	3.2	13.8	3.2	12.1
5	7.5	16.8	7.0	11.8	6.0	10.5	6.7	16.2
6	5.4	10.8	4.8	8.7	5.7	8.2	6.2	16.5
7	4.3	8.8	4.1	8.3	5.2	8.0	6.3	17.0
8	3.8	7.5	3.9	8.2	4.1	9.2	6.5	17.7
9	3.2	7.2	3.2	8.1	3.6	10.1	6.0	16.8
10	3.2	7.1	3.2	8.2	3.4	10.6	6.2	17.3

Table of Total Nitrogen per Segment (µgs.) and Standard Error

	S1		S2		S3		S4	
	Total N	S.E.	Total N	S.E.	Total N	S.E.	Total N	S.E.
Day 3	25.5	1.4	18.0	1.1	12.8	0.9	13.8	0.8
4	31.2	1.8	23.9	1.3	17.0	0.9	15.3	1.0
5	24.3	1.6	18.8	1.2	16.5	1.2	22.9	1.2
6	16.2	1.1	13.5	1.0	13.9	1.1	22.7	1.1
7	13.1	1.0	12.4	0.8	13.2	1.0	23.3	1.0
8	11.3	0.9	12.1	0.8	13.3	1.0	24.2	1.3
9	10.4	0.7	11.3	0.5	13.7	1.1	22.8	1.2
10	10.3	0.7	11.4	0.6	14.0	0.8	23.5	1.0

The table below gives the values for total nitrogen per cell in $\mu g. \times 10^5$.

	S1	S2	S3	S4
Day 3	11.86	15.74	23.81	42.47
4	13.71	18.21	32.68	50.58
5	15.75	23.72	42.85	76.33
6	34.11	41.65	43.44	74.43
7	33.59	38.75	41.58	74.56
8	32.74	37.52	42.56	76.82
9	32.00	36.16	42.48	73.54
10	31.45	35.91	44.80	76.42

The tables below give the values for chlorophyll a (Ca), chlorophyll b (Cb) and total chlorophyll per segment. The values for standard error of total chlorophyll are also given. All values are in mgs. x 10^4.

Table Showing Chlorophyll a and Chlorophyll b per Segment

	S1		S2		S3		S4	
	Ca	Cb	Ca	Cb	Ca	Cb	Ca	Cb
Day 3	2.223	1.281	4.569	1.846	5.912	2.034	8.228	3.190
4	3.532	1.357	4.820	1.844	6.372	2.230	36.824	9.180
5	3.554	2.021	4.984	1.992	7.028	2.418	41.220	10.636
6	4.168	2.204	6.702	2.526	7.236	2.942	42.356	14.275
7	4.705	2.560	6.760	2.854	10.676	3.866	42.820	15.746
8	9.099	3.291	13.664	4.212	16.348	5.632	43.836	15.872
9	14.656	5.992	18.584	7.700	24.564	9.360	43.968	16.120
10	17.316	6.501	22.608	7.794	26.140	9.996	44.510	16.544

Table Showing Total Chlorophyll per Segment

	S1		S2		S3		S4	
	Total C	S.E.	Total C	S.E.	Total C	S.E.	Total C	S.E.
Day 3	3.504	0.29	6.415	0.68	7.946	0.80	11.418	0.96
4	4.889	0.34	6.664	0.65	8.602	0.94	46.624	3.85
5	5.575	0.60	6.976	0.69	9.446	0.83	51.856	4.24
6	6.372	0.72	9.228	0.80	10.178	1.10	56.631	4.54
7	7.265	0.70	9.614	0.84	14.542	1.14	58.566	5.01
8	12.390	1.10	17.276	1.71	21.970	1.74	59.708	4.76
9	20.648	2.41	26.284	2.34	33.924	2.76	60.088	5.21
10	23.817	2.30	30.402	3.00	36.136	3.58	61.054	4.43

The table below gives the values for total chlorophyll per cell.

Chlorophyll Content in μg. x 10^8

Day	S1	S2	S3	S4
3	0.16	0.56	1.48	3.51
4	0.21	0.51	1.65	15.41
5	0.36	0.88	2.45	17.29
6	1.34	2.86	3.18	18.67
7	1.86	3.00	4.58	18.74
8	3.59	5.36	6.81	18.96
9	6.35	8.41	10.52	19.38
10	7.27	9.58	11.56	19.85

The tables below give the values for R.N.A. content per segment per cell and per µg. protein. A table of standard error (S.E.) for the primary data is also given.

R.N.A. per Segment and Standard Error

	S1		S2		S3		S4	
Day	µgs.	S.E.	µgs.	S.E.	µgs.	S.E.	µgs.	S.E.
3	17.60	1.21	9.90	0.86	6.16	0.56	4.40	0.24
4	13.31	0.94	11.00	0.93	6.71	0.63	3.96	0.20
5	9.24	0.73	7.26	0.74	5.61	0.60	4.07	0.31
6	5.39	0.64	4.40	0.54	3.74	0.41	2.86	0.21
7	2.53	0.30	3.30	0.21	3.41	0.24	1.87	0.22
8	2.09	0.28	3.08	0.30	2.09	0.18	2.20	0.14
9	2.09	0.29	1.76	0.10	2.20	0.19	2.09	0.11
10	0.99	0.10	1.21	0.22	1.21	0.10	1.21	0.12

R.N.A. per Cell µg. x 10^5 and R.N.A. per µg. protein x 10^2

	S1		S2		S3		S4	
Day	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell
3	13.98	8.16	11.24	8.67	10.06	11.46	6.40	13.54
4	8.38	5.85	9.21	8.38	7.78	12.87	5.24	13.06
5	8.80	5.98	10.17	9.15	8.55	14.60	4.02	13.57
6	7.99	11.35	8.09	13.67	7.29	11.99	2.77	9.15
7	4.60	6.48	6.36	10.35	6.82	10.70	1.76	5.98
8	4.46	6.08	6.00	9.57	3.63	6.69	2.01	6.97
9	4.64	6.43	3.48	5.63	3.49	6.84	1.99	6.76
10	2.23	3.02	2.36	3.81	1.83	3.87	1.12	3.95

The tables below give the values for respiratory activity per segment per cell and per µg. protein. A table of standard error (S.E.) for the primary data is also given.

a) Respiratory activity per segment expressed as µl. Oxygen absorbed per hr.

	S1		S2		S3		S4	
	µl. O_2	S.E.	µl. O_2	S.E.	µl. O_2	S.E.	µl. O_2	S.E.
Day 3	3.97	0.32	3.84	0.41	2.69	0.25	1.86	0.04
4	3.60	0.31	3.59	0.38	2.31	0.21	1.54	0.03
5	3.10	0.28	2.48	0.21	1.92	0.12	1.49	0.04
6	1.61	0.14	1.61	0.14	1.67	0.10	1.86	0.05
7	1.24	0.16	1.24	0.14	1.30	0.04	1.74	0.04
8	0.99	0.09	0.87	0.06	1.03	0.02	1.20	0.07
9	0.87	0.10	1.12	0.08	1.15	0.05	1.49	0.04
10	0.99	0.12	1.12	0.09	1.03	0.06	1.36	0.02

b) Respiratory activity per cell expressed as µl. $O_2 \times 10^5$ absorbed per hr. and activity per µg. protein as µl. $O_2 \times 10^2$ absorbed per hr.

	S1		S2		S3		S4	
	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein
Day 3	1.84	3.15	3.35	4.36	5.00	4.39	5.72	2.70
4	1.58	2.26	2.74	3.01	4.44	2.68	5.09	2.04
5	2.01	2.95	3.13	3.36	4.99	2.93	4.97	1.47
6	3.38	2.38	4.99	2.96	5.22	3.26	6.10	1.80
7	3.18	2.25	3.86	2.39	4.09	2.60	5.57	1.64
8	2.87	2.11	2.70	1.70	3.30	1.79	3.81	1.08
9	2.68	1.93	3.47	2.21	3.57	1.82	4.81	1.42
10	3.02	2.23	3.42	2.18	3.30	1.55	4.42	1.26

The tables below give the values for photosynthetic activity per segment, per cell and per µg. protein. A table of standard error (S.E.) for the primary data is also given.

a) Photosynthetic activity per segment expressed as µl. O_2 liberated per hr.

	S1		S2		S3		S4	
	µl. O_2	S.E.	µl. O_2	S.E.	µl. O_2	S.E.	µl. O_2	S.E.
Day 3	1.67	0.11	2.30	0.14	1.41	0.15	1.22	0.16
4	1.80	0.14	2.82	0.20	1.80	0.19	1.54	0.08
5	2.08	0.16	2.09	0.18	1.65	0.18	1.62	0.09
6	0.97	0.10	1.35	0.16	1.40	0.10	1.73	0.11
7	0.86	0.07	0.85	0.17	1.03	0.05	1.74	0.12
8	0.86	0.06	1.13	0.08	0.90	0.04	1.33	0.14
9	0.61	0.04	1.12	0.06	1.02	0.07	1.49	0.07
10	0.73	0.06	0.99	0.04	1.03	0.04	1.49	0.06

b) Activity per cell expressed as µl. $O_2 \times 10^5$ liberated per hr. and per µg. protein as µl. $O_2 \times 10^2$ liberated per hr.

	S1		S2		S3		S4	
	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein
Day 3	0.77	1.33	2.01	2.61	2.62	2.30	3.75	1.77
4	0.79	1.13	2.15	2.36	3.46	2.09	5.09	2.03
5	1.35	1.98	2.64	2.83	4.29	2.52	5.40	1.60
6	2.04	1.44	4.19	2.48	4.38	2.73	5.67	1.68
7	2.21	1.56	2.66	1.64	3.24	2.06	5.57	1.64
8	2.49	1.83	3.50	1.97	2.88	1.57	4.22	1.20
9	1.88	1.36	3.58	2.21	3.16	1.62	4.81	1.42
10	2.23	1.64	3.22	1.93	3.30	1.55	4.85	1.38

The tables below give the values for invertase activity per segment, per cell and per µg. protein. A table of standard error (S.E.) for the primary data is also given.

a) Invertase activity per segment expressed in µ Moles reducing sugar produced per hr.

	S1		S2		S3		S4	
	µ M.	S.E.	µ M.	S.E.	µ M.	S.E.	µ M.	S.E.
Day 3	1.43	0.23	4.01	0.41	8.96	0.75	4.95	0.36
4	2.71	0.30	6.52	0.48	11.63	0.85	4.42	0.31
5	2.80	0.31	9.93	0.67	12.13	0.82	5.53	0.34
6	8.01	0.50	14.75	1.10	13.99	0.96	7.37	0.44
7	9.44	0.48	8.10	0.74	7.69	0.70	4.35	0.25
8	5.60	0.24	4.83	0.30	5.94	0.31	2.78	0.22
9	5.55	0.22	3.55	0.26	4.94	0.24	2.28	0.20
10	5.00	0.36	3.11	0.31	4.50	0.20	2.11	0.18

b) Invertase activity per cell expressed in µ Moles reducing sugar x 10^5 produced per hr. Invertase activity per µg. protein expressed in µ Moles reducing sugar x 10^2 produced per hr.

	S1		S2		S3		S4	
	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein
Day 3	0.66	1.14	3.50	4.55	16.67	14.63	15.23	7.20
4	1.19	1.71	4.97	5.46	22.36	13.48	14.61	5.85
5	1.82	2.67	12.53	13.46	31.51	18.49	18.43	5.46
6	16.86	11.87	45.74	27.11	43.72	27.27	24.16	7.15
7	24.21	17.16	25.31	15.61	24.22	15.38	13.92	4.09
8	16.23	11.94	14.98	9.42	19.00	10.33	8.83	12.51
9	17.08	12.33	11.36	7.02	15.32	7.83	7.35	2.17
10	15.28	11.26	9.80	6.06	14.40	6.79	6.86	1.95

The tables below give the values for proteolytic enzyme activity per segment, per cell and per µg. protein. A table of standard error (S.E.) for the primary data is also given.

a) Proteolytic activity per segment expressed as µg. Tyrosine equivalents produced per hr.

	S1		S2		S3		S4	
	µg. Tyr.	S.E.	µg. Tyr.	S.E.	µg. Tyr.	S.E.	µg. Tyr.	S.E.
Day 3	25.0	1.6	19.2	1.3	13.6	0.9	13.6	1.2
4	19.5	1.1	19.5	1.6	15.0	1.4	13.0	0.7
5	17.0	1.2	15.0	1.4	15.6	1.2	17.4	1.6
6	8.8	0.9	11.5	1.1	12.6	1.3	18.0	1.4
7	7.9	0.6	8.5	0.9	10.1	0.6	11.0	0.8
8	7.4	0.6	8.0	0.7	8.8	0.4	10.2	0.8
9	77.0	0.3	7.5	0.7	7.2	0.2	9.6	0.6
10	7.5	0.5	8.5	0.7	7.2	0.4	9.0	0.6

b) Activity per cell expressed as µg. Tyrosine equivalents x 10^4 produced per hr., and activity per µg. protein expressed as µg. Tyrosine equivalents x10 per hr.

	S1		S2		S3		S4	
	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein
Day 3	1.16	1.99	1.68	2.18	2.53	2.22	4.18	1.98
4	0.86	1.23	1.49	1.63	2.88	1.73	4.30	1.72
5	1.10	1.62	1.89	2.03	4.05	2.38	5.80	1.72
6	1.85	1.30	3.57	2.11	3.94	2.46	5.90	1.75
7	2.03	1.44	2.67	1.64	3.18	2.02	3.52	1.03
8	2.14	1.58	2.48	1.56	2.82	1.53	3.24	0.92
9	2.15	1.56	2.40	1.48	2.23	1.14	3.10	0.91
10	2.29	1.69	2.68	1.66	2.30	1.09	2.93	0.83

The tables below give the values for phosphatase activity per segment, per cell and per µg. protein. A table of standard error for the primary data is also given.

a) Activity per segment, expressed in µg. inorganic phosphorus (Pi) produced per hr.

	S1		S2		S3		S4	
	µg. Pi	S.E.	µg. Pi	S.E.	µg. Pi	S.E.	µg. Pi	S.E.
Day 3	11.24	0.94	10.90	0.91	6.40	0.81	5.96	0.31
4	9.48	0.76	11.24	1.02	6.72	0.82	6.20	0.24
5	15.00	1.28	18.94	1.56	10.60	0.96	10.66	0.74
6	11.10	1.00	7.80	0.82	7.92	0.80	7.50	0.61
7	6.80	0.64	6.40	0.80	7.66	0.71	6.80	0.41
8	5.96	0.54	6.28	0.71	7.40	0.43	6.60	0.33
9	5.88	0.52	5.74	0.44	6.80	0.40	5.50	0.20
10	6.00	0.71	5.86	0.43	6.74	0.52	4.90	0.22

b) Activity per cell, expressed in µg. Pi hr. x 10^4 and activity per µg. protein expressed in µg. Pi hr. x 10^2.

	S1		S2		S3		S4	
	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein	per cell	per µg. protein
Day 3	0.52	8.93	0.95	12.37	1.19	10.45	1.83	8.67
4	0.42	5.97	0.86	9.41	1.29	7.79	2.05	8.20
5	0.97	14.29	2.39	25.66	2.75	16.16	3.55	10.52
6	2.34	16.44	2.42	14.34	2.48	15.44	2.46	7.27
7	1.74	12.36	2.00	12.33	2.41	15.32	2.18	6.40
8	1.73	12.71	1.95	12.10	2.37	12.87	2.10	5.97
9	1.81	13.07	1.84	11.34	2.11	10.78	1.77	5.24
10	1.83	13.51	1.85	11.42	2.16	10.17	1.59	4.53

APPENDIX II

Culture Experiments

The comparison of plants or plant organs grown in culture with those grown normally has attracted many authors. However, attempts to culture monocotyledonous plants have so far met with little success. The successful attempts have been confined mainly to the culture of the whole embryo of various cereals. The first report was that of Brown and Morris (1890) who cultured a variety of embryos of the Gramineae. De Ropp (1939) on rye and James and James (1940) on barley also reported successful cultures of excised embryos. In a series of papers, Brown. R (1943a, 1943b, 1946) investigated various differences in the growth of excised barley embryos in culture with those grown from whole seed. De Ropp (1939) stated the embryo is a complete biological system although its growth is modified when excised and grown in culture. In 1945, the same author tackled the problem of growing in culture a single organ. He chose the stem tip of rye and found some limited growth, various vitamins and 'growth promoting' substances were added to the culture medium to no good effect. He found, however, that if a root developed by chance, immediate meristematic activity and growth resulted. He suggested that the usual lack of growth was due to the leaf being incapable of absorbing nutrient for itself. De Ropp was using an agar medium from which absorption of nutrient by a leaf would probably be more difficult than from a liquid medium. The limited growth achieved by the stem tips was found to be solely due to cell extension (de Ropp 1946) no mitotic figures ever being found in the cultured material.

Since White (1933) had reported some divisions in isolated stem tips of Stellaria media in culture de Ropp also decided to try a dicotyledon and chose disks from the inner leaves of cabbage as his material to take advantage of a certain degree of natural sterility. Apart from some callus growth at the cut edges of the leaf disks no cell divisions again occurred. De Ropp (1947).

Monocotyledonous tissue in general, with the exception of root tissue, and dicotyledonous tissue appeared to be unsuitable for culturing. There was, however, one notable exception that of Loo (1945) who succeeded in culturing the stem tips of asparagus. His cultures were of apparently unlimited growth and produced neither roots nor callus tissue. Cultures of rather more specialised tissues of monocotyledons were reported by La Rue (1949) Straus and La Rue (1954) and Straus (1954) who successfully cultured maize endosperm and Norstog (1956) with rye grass endosperm. It was pointed out that the culturing of endosperms was not indicative of exceptional culturability of the tissue type as La Rue and his students had failed with many other endosperms. Morel and Wetmore in their search for a culturable monocotyledonous tissue succeeded in 1951 in culturing cubes cut from the root tubers of two tropical members of the Araceae. A good growth of callus was achieved and also the production of buds and roots.

Hildebrandt and Co. (1963) grew many different green tissues in culture but they were interested primarily in the production of callus and although they were successful in producing callus from excised bits of leaf petiole, they did not report having done so with the lamina.

As previously mentioned, root tissue appears to be the only generally culturable monocotyledonous tissue and there have been many reports of this. On some occasions, apparently, unlimited growth was achieved. This work has recently been reviewed by Butcher and Street (1964). Success has also been often achieved with whole excised embryos and this work has been reviewed by Narayanaswami and Norstog (1964).

The work described below was undertaken mainly to try and achieve full cell extension of the basal region of the leaf in culture rather than the faint hope of achieving cell division. It was hoped that some interesting differences would arise between the mature leaves grown in culture and those grown normally.

Methods

One of the principal difficulties encountered in attempts to culture leaf material is that of sterility. It was, therefore, decided to try only short-term culturing. A reasonable degree of asepsis would be observed to prevent bacterial infection from reaching the massive level.

Seeds were sterilised in the normal manner with calcium hypochlorite and methylated spirit and germinated on previously autoclaved filter paper in sterile water. The vermiculite, dishes, media and all instruments used were autoclaved, any surfaces in contact with the leaves during harvesting and segmenting were sterilised with methylated spirit and these operations were carried out in a sterile room.

Media

Preliminary experiments were carried out using Haberlandt's medium with 2% sucrose. A simple medium containing 4% sucrose and 300 mgs. per litre potassium chloride as used by Brown. R (1943a) was tried with and without the addition of 5 p.p.m. gibberellic acid.

The medium of Bonner and Addicot (1937) which is commonly used in this department for the culture of pea roots was tried by itself and in combination with 15% coconut milk and/or 5x 10^{-7}M. naphthyl acetic acid (N.A.A.).

10 ml. of media were used in all cases and the segments were allowed to float on the media in Petri dishes. On one occasion, the segments were placed on sintered glass disks in the Petri dishes so that they would be in contact with the medium and yet have only slight obstruction to their gaseous exchange. However, the segments tended to curl up out of the media when placed on the disks so these were abandoned.

All incubations were carried out in the light in the same growth room as the wheat was grown in.

RESULTS

Experiment (1)

Medium: Haberlandt's in 2% sucrose

A batch of five 72 hr. 3.5 cm. leaves was harvested and placed in the medium under semi-sterile conditions as described above. All segments were 5 mm. long at 72 hrs. and were remeasured at 96 hrs. After measuring the segments were placed in 5% chromic acid for cell counts.

Increases in length were found in S1 and S2 only. In S1 the lengths at 96 hrs. ranged from 8.5 mm. to 9.5 mm. and in S2 from 6 to 8 mm.

The cell numbers found were recorded in Table I below in comparison with the previously found mean value for the 72 hr. leaf.

TABLE I

Cell numbers per segment

Segment No.	72 hrs. (mean)	96 hrs. (cultured)
1)	210,312	239,899
2)	86,562	96,668
3)	41,265	53,178
4)	34,833	34,687
5)	33,271	34,468
6)	32,167	31,875
7)	24,615	23,178

There is a slight, but possibly genuine, increase in cell number in the first three segments. If this is genuine it is suggested that it would most likely have been due to cells that were already in some stage of division at 72 hrs. completing their cycle. The increases in length of the first two segments was far less than that which would have occurred in normal growth. Taking the previously calculated figure of 60,000 cells per cm. in the mature leaf, it should be possible for the cultured first segment containing 240,000 cells to attain a length of 4 cms.

Experiment (2)

Medium: 4% sucrose and potassium chloride (300 mgs./litre), with and without 5 p.p.m. gibberellic acid (G.A.).

A batch of six 72 hr. leaves were taken but only the basal four segments were used in the experiment as it seemed unlikely that the terminal three would grow at all.

The segments were divided into two batches of three each. One batch was cultured in the medium containing G.A. and the other without it. The segments were weighed before placing in the medium. After 24 hrs., the segments were measured, reweighed and placed in chromic acid for cell counts. The results were recorded in Table II.

TABLE II

	-G.A.	Lengths of segments	+G.A.	
	72 hrs.	96 hrs.	72 hrs.	96 hrs.
1)	5,	10, 10, 9.	5,	11.5, 11, 10.
2)	5,	7, 7, 6.5.	5,	8.5, 7.5, 7.
3)	5,	5, 5, 5.	5,	5, 5, 5.
4)	5,	5, 5, 5.	5,	5, 5, 5.
		Ave. fresh weigh mgs. per segment		
1)	1.60	3.00	1.67	3.00
2)	1.90	2.43	1.93	2.83
3)	2.13	1.83	1.93	1.87
4)	2.27	1.77	2.30	1.97
		Ave. cell number per segment		
1)	210,312	190,892	210,312	204,159
2)	86,562	86,104	86,562	82,083
3)	41,265	46,700	41,265	44,791
4)	34,833	36,247	34,833	33,948

A considerably greater increase in length was found than had occurred with Haberlandt's medium, the increase with G.A. was greater than without but the difference is not significant. The fresh weight increase was slightly less than might have been expected to accompany the increases in length in the first two segments. There was a substantial loss of fresh weight from S3 and S4, the most likely explanation for this is respiratory loss, the segments being unable to utilise the sucrose in the medium.

There was no significant change in cell number, the slight increase found in the previous experiment was not repeated.

The next experiments were designed to find out to what extent the cells of the meristematic segments would elongate under cultural conditions, as it seemed unlikely that cell division would be achieved. So far segments had only been cultured for 24 hrs. to avoid bacterial contamination but it was now necessary to maintain the cultures rather longer. To improve sterility, it was decided to immerse the seedling, after harvesting, in saturated calcium hypochlorite solution, the depth of this solution to be slightly less than the height of the coleoptile, the seedlings would then be rinsed in sterile distilled water. The coleoptile would probably be seriously damaged by this treatment but it was hoped that the first leaf underneath would be unharmed and naturally sterile. The segments were cut with the coleoptile on, only the basal two being taken, and placed in the medium.

The basic medium used was Bonner and Addicott's as it was thought that a full medium would be advantageous for longer culturing. Coconut milk and N.A.A. were also added to some replicates, giving four different media: 1) Basic medium, 2) Medium + N.A.A., 3) Medium + coconut milk, 4) Medium + N.A.A. + coconut milk.

In the first experiment, no growth had been achieved after 58 hrs. and the experiment was abandoned. It was thought that the calcium hypochlorite treatment had killed the segments. Another experiment was, therefore, set up with a less severe method of sterilisation of the coleoptile, a two minute immersion in Methylated spirit. On this occasion, slight growth was observed in two of the five replicates of S2 in the basic medium. No growth occurred in S1 in any of the treatments, again the sterilisation treatment was thought responsible and it was decided to abandon it. In the next experiment, the coleoptile was not sterilised and there was still no growth except for occasional slight growth in S2. It seemed unlikely that the media were responsible for the lack of growth as extension had been achieved with Hildebrandt's medium and with sucrose and KCl. Previously, extension had also been observed in segments which had been used for respiration experiments and had been allowed to remain in Warburg flasks containing water overnight. Since the only difference between the method of the last culture experiment and the early ones was the presence of the coleoptile this was considered to be responsible.

The segments for the experiment described below were prepared in the original manner without coleoptiles.

Experiment 3)

Media 1) Bonner and Addicott's (B.&A's), 2) B. and A's + coconut milk 3) B. and A's + N.A.A., 4) B. and A's + coconut milk + N.A.A.

The basal two segments of 24 x 72 hr. leaves were cut and placed in the above media, six segments per dish. At 96 hrs., three of the segments were removed, measured, weighed and placed in chromic acid for cell counts. The remaining three were treated similarly at 120 hrs. The results were recorded in Table III below:

TABLE III

		Fresh Weight mgs. per segment			Cell number per segment		
Medium	Segment	72 hrs.	96 hrs.	120 hrs.	72 hrs.	96 hrs.	120 hrs.
1	1	1.45	3.43	3.90	210,312	234,375	217,708
	2	1.65	2.93	3.00	86,562	69,792	100,000
2	1	1.45	3.40	4.80	210,312	230,000	242,250
	2	1.65	3.00	3.23	86,562	110,410	96,666
3	1	1.45	3.93	4.50	210,312	241,667	236,458
	2	1.65	3.13	3.37	86,562	78,125	76,042
4	1	1.45	3.60	4.10	210,312	230,000	231,250
	2	1.65	3.10	2.97	86,562	90,625	95,833
		Lengths of Segments					
1	1	5	9, 8, 9.	9.5, 10, 10.			
	2	5	6, 6.5, 7.	6.5, 6.5, 7.			
2	1	5	8.5, 8.5, 8.	10.5, 10, 8.			
	2	5	6.5, 6.6.	6.5, 6.5, 7.			
3	1	5	8.5, 9, 8.	10, 11, 9.			
	2	5	7, 7, 6.5.	7, 7, 7.			
4	1	5	8, 8, 8.5	10, 9, 10.			
	2	5	6.5, 6.5, 7.	6.5, 6.5, 7.			

At 120 hrs., some of the cultures were heavily infected with bacteria and the weights found then cannot be considered reliable. There was no increase in cell number and the increases in segment length took place almost entirely in the first 24 hrs. of culture.

Had the cultures remained sterile it is possible that further extension would have occurred. The extension inhibiting effect of the coleoptile may have been caused by obstructing the respiration of the leaf or by the release of some inhibiting substance into the medium. Neither coconut milk nor N.A.A. had any great effect on the growth of the segments.

At this stage, the experiments were abondoned as it did not seem likely that either cell division or complete extension of the segments would be possible in culture.

Since this work was concluded, there has been one report of successful growth in culture of monocotyledonous shoots. Smith (1966) personal communication grew excised embryo shoot apices of wheat on a semi-liquid standard medium with 2% sucrose and G.A. She found the rate of growth was greater in explants in which roots were formed.